Remarks

Claims 21-32 are pending. Claims 21-25 and 27 are amended herein. It is believed that these amendments add no new matter. In light of these amendments and the following remarks, applicant respectfully requests entry of these amendments, reconsideration of this application, and allowance of the claims.

Amended claims 21, 23, 25 and 27 now recite use of the claimed mutation in any APP isoform, for example, the isoforms disclosed in the carryover paragraph on page 2 of the Specification, and in the first full paragraph on page 11. Claims 21, 23, 25 and 27 are also amended to more accurately recite the present invention. Amended claims 22 and 24 more distinctly claim the usefulness of the invention. Support can be found in the specification on page 20, lines 24-25. No new matter is believed added by these amendments, and their entry is respectfully requested.

Double Patenting

Claims 25-28 remain rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 11 and 12 of U.S. Patent No. 5,455,169. The Office states that although the conflicting claims are not identical, they are not patentably distinct from each other because the specific nucleic acid sequences claimed in claims 11 and 12 of the '169 patent are encompassed within the scope of claims 25-28 in the instant claims.

While applicant does not concede the Examiner's position, a Terminal Disclaimer in compliance with 37 C.F.R. § 1.321(c) is submitted herewith. Applicant therefore respectfully asserts that this terminal disclaimer effectively renders moot any obviousness-type double patenting rejections relating to U.S. Patent No. 5,455,169. By submitting this terminal disclaimer, it is understood that applicant does not admit that obviousness-type double patenting exists in this case.

35 U.S.C. § 112, first paragraph

Claims 21-24, 29 and 30 remain rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. After summarizing applicant's previous arguments regarding the Office's allegation of lack of enablement, the Office states that "the means through which to produce any transgenic animal at the time of the present invention, 1992, is not the issue. The issue turns on whether or not the specification provides guidance on producing transgenic animal that has a use in the art as taught by the specification." The Office goes on to state that applicant's specification states that the transgenic animal can be used to study the progression of Alzheimer's disease, to identify treatments and drugs that alter the degenerative progress, arrest the development or reverse the progression of Alzheimer's disease, and to identify environmental factors that affect the onset of Alzheimer's disease (specification, page 4, parag. 3 and 4). At page 5, lines 10-20, the Office goes on to state that

> [t]his use requires that the animal develop a pathogenesis specific for Alzheimer's disease. The expression of the encoded protein would not be so specific. Even the development of amyloid plaques would not rise to the level of an enabled use for the animal as at the time of filing amyloid plaques were known to develop in aged humans and aged monkeys (Selkoe, page 432, col. 1, parag. 3, lines 1-10). Unless the animal continues from β-amyloid plaque formation to more Alzheimer's disease related pathologies, the animal would not have a use under applicant's disclosure. While applicant's specifically disclosed mice may express human APP, it is the development of Alzheimer's related pathologies that is unpredictable and is what is required for use in studying Alzheimer's disease progression and factors that affect progression or cause inhibition of Alzheimer's disease.

The foregoing required use is impermissibly arbitrary because it is substantially narrower than what the specification teaches. The specification describes many credible, substantial and specific uses for transgenic animals expressing the Swedish mutation, and a great variety of such animals have been actually produced according to the specifically disclosed embodiments.

Further, there is no legal basis for the Office to arbitrarily determine what the use of applicant's invention must be. "...[W]hen a compound or composition claim is not limited by a recited use, any enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use." (Underline added.) M.P.E.P. § 2164.01(c). Thus, the Office has failed to make a *prima facie* case of lack of enablement because applicant provides at least one use of the claimed invention, e.g., studying the progression of Alzheimer's Disease. As noted above, the Office acknowledges that applicant's specification states this as a use of the claimed non-human transgenic animal. The Office says that applicant should point to a place in the specification where there is disclosure that the animal "can be used to assay factors affecting APP processing." See page 6, lines 19-20 in the Office Action.

We respectfully observe that the Office Action dated March 10, 2003 is fundamentally flawed in the foregoing regard. The premise of the rejection, as stated on page 5, is that certainly the transgenic animals can be made, but the issue of enablement "turns upon whether or not the specification provides guidance on producing a transgenic animal that has a use in the art as taught by the specification." Therefore, the Office acknowledges that the issue of the present rejection is one of "how to use," not "how to make" the mice. The Office now asserts that the only permitted utility is just as disclosed on page 4 of the Specification in paragraphs 3 and 4. The Office presumption is unwarranted that "[t]his use requires that the animal develop a pathogenesis specific for Alzheimer's." Although not specifically stated, it seems also that the Office believes the claims read upon embodiments that are inoperable for performing the taught utility, particularly, because there is suggestion in some of the relevant literature that the 695 isoform under control of the NSE promoter may not necessarily develop neuropathological characteristics that are specific to Alzheimer's. We disagree with the Office assessment and presumption, and so respectfully traverse the rejection.

At the essential core of the enablement rejection stated on page 5 lies a faulty presumption. This presumption is that any possible utility for the transgenic animals inherently requires the transgenic animals to "develop a pathogenesis specific for Alzheimer's." Even if the

transgenic animals are shown to produce the Alzheimer's-characteristic β-amyloid plaques, this is asserted to be insufficient because such plaques are nonspecific to Alzheimer's since they also appear in Down's patients, aged monkey and dogs. Neither the applicant nor the art has anywhere stated that, to be useful, the transgenic animals must essentially develop specific end-stage neuropathological features of Alzheimer's as recited by the Office. Though many of them do develop such features, it is at most a preferred feature of the claimed transgenic animals, as stated on page 9 at lines 20-21.

We fail to understand how the Office has now elevated a preferred feature of an animal model to the status of inoperability or how-to-use lack of enablement. Applicant has disclosed other utilities in addition to those recited on page 4. By way of example, see the underlined heading on page 20 at line 16 of the Specification, "Model Animals and Cell Lines." The paragraph at lines 18-26 recites utilities not considered by the Examiner, namely, use as animal model systems for screening for drugs and evaluating drug effectiveness, and a tool for defining the biochemistry of APP and β-amyloid metabolism. These do not require the specific pathogenesis as defined by the Office. It is clear error that the Office presumes that a particular stage of pathogenesis is required, at least because model utility is also asserted in the Specification.

What is an animal model? It is not necessarily one that develops neuropathological features specific only to Alzheimer's. The attached excerpt from Dorland's Illustrated Medical Dictionary (27th ed.) W.B. Saunders & Co. (1988) (Exhibit A) shows that an animal model is comprised of any condition found in an animal that is of value in studying a biological phenomenon, e.g., a pathological mechanism of an animal disorder. In this context, it is sufficient that the transgenic animal translates the claimed mutant mRNA because the expression of mutant mRNA is sufficient for studying a biological phenomenon of APP expression. It is further sufficient that the animal expresses the mutant form of human APP. It is merely preferred that the APP reacts *in vivo* to form β-amyloid. It is, also, only preferred that the β-amyloid accrete and then agglomerate into plaques, as accretion and agglomeration of plaques are both neuropathological conditions of Alzheimer's. It is, further, only preferred that the

plaques are associated with hyperphosphorlyated tau which is associated with tangle formation in AD. Applicant has disclosed the model-utility, and this does not require full specific Alzheimer's pathogenesis as presumed by the Office. It is error to now impose a standard that, in effect, requires that each and every utility to which the claimed transgenic animals can be applied must relate to full-blown or specific Alzheimer's pathology, or else the claims are nonenabled. It is sufficient that a model may be used to test a biological condition, such as the expression level of mRNA or mutated APP, or the biochemistry of amyloid precursor protein (APP) or β-amyloid metabolism, which are very important to the understanding of this disease. Furthermore, even if plaques are nonspecific as the Office asserts, they are also characteristic of an Alzheimer's pathology and can be used as a model. If the Office has a question about enabled utility, it is sufficient for Applicant to submit pertinent references showing the truth of statements that the Office thinks are untrue. The crux of the present enablement dispute centers upon an observance that three references, Malherbe et al., Quon et al, and Higgins et al. report transgenic mice incorporating the APP695 isoform under control of the NSE promoter failed to develop plaques as a measure of neuropathological conditions of Alzheimer's. All of these references confirm that APP751 isoforms under control of the NSE promoter did develop β-amyloid plaques. The issue is relevant because pages 23-25 of the Specification describe use of neuronal promoters operably linked to APP isoforms, and the NSE, NFH and Thy-1 promoters linked to APP751 on pages 24-25.

An attached article (Exhibit B), Pierrat et al., "Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology," 94 Proc. Nat'l Acad Sci. 13287-13292 (1997), describes three lines of transgenic mice expressing the claimed 670/671 (KM>NL) mutation under control of the Thy-1 promoter. One line, APP 14, expressed mutant APP751 2-fold over endogenous expression of APP under control of the human Thy-1 promoter, but did not form plaques. Another line, APP 22, expressed mutant APP751 2-fold over endogenous expression of APP under control of the human Thy-1 promoter and did form plaques where additionally the V717I mutation was inserted. A third line, APP 23, expressed mutant APP751 (K670M/N671L) under control of the murine Thy-1 promoter and did form plaques. The APP

23 line under control of the murine promoter overexpressed the transgene by 7-fold over endogenous expression of APP. See Pierrat et al., p. 13288, column 2. In addition to plaques, the APP 22 and APP 23 mice show inflammatory processes reminiscent of Alzheimer's (p. 13289, column 1), neuritic loss and distortion of cholinergic fibers in the vicinity of plaques (p. 13289, column 2), and hyperphosphorylated tau (p. 13290).

Applicant repeatedly makes note of the advantages of combining the 670/671 mutation with the 717 mutation, for example, in the last paragraph on page 22 of the Specification. Even so, as confirmed by Hsiao et al. and others, use of the 717 mutation is not required. Applicant also clearly teaches the Thy-1 promoter (pThy751), described in the specification on page 25, lines 7-13.

The discussion in Pierrat et al. on pages 13290-13291 confirms that exceeding a threshold expression of human APP with a FAD-associated mutation is the trigger for plaque-like pathology. Pierrat et al. reports that the APP22 mice exceeded this threshold at 200% of endogenous expression and formed plaques, while the APP 14 mice expressing at this level did not develop plaques. Hsiao et al. reports having exceeded the threshold by expressing at 300% and also at 600% of endogenous APP levels, and Games et al. at 700%. Hsiao et al. discusses threshold issues in the Background section thereof, indicating a useful range from generally upwards of 150%. Thus, it is the general opinion of those skilled in the art that the overall expression level is responsible for plaque formation—not, as the Office asserts, Hsiao's use of a prion promoter in combination with the 695 isoform.

The art teaches that the precise level of threshold for plaque formation differs for each strain of mice and the type of mutation that may be employed. The general point is that an expression threshold is exceeded, i.e., a natural mechanism for ridding tissue of β-amyloid is overwhelmed. Furthermore, all isoforms tested thus far have been shown to develop plaques in transgenic mice. For example, other references of record (Quon et al, Higgins et al., Malherbe et al.) have reported plaque formation using the 751 isoform under control of the NSE promoter. Hsiao et al. reports plaque formation using the 695 isoform under control of a prion promoter.

In any case, the combination of Thy-1 and the 670/671 and 717 mutations, as taught in the specification, were confirmed by Peirrat et al. to produce useful mice. Although this mouse was confirmed in Pierrat et al. to form plaques, for all of the reasons stated herein, Applicant does not concede that plaque formation is required to have a useful animal.

What is important and unique about the preferred 670/671 mutation is that, when similar expression levels of APP are compared between mutated and nonmutated forms of APP, the mutation results in significantly increased levels of plaque-forming β-amyloid. Applicant noted this use of the mutation at an interesting cleavage location in the specification from page 14 at line 29 to page 15 at line 26. Others have confirmed and reported the truth of this assertion and its utility by experimentation. An attached Abstract (Exhibit C), Citron et al., *Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production*, Nature 1992 Dec. 17; 360 (6405):672-4 shows that the 695 isoform of the 670/671 mutation produces 6- to 8-fold more beta-APP than is obtained from similar expression levels using the non-mutated isoform. "The Met 596 to Leu mutation is principally responsible for the increase. These data establish a direct link between a FAD genotype and the clinicopathological phenotype." Thus, the specification teaches and the art confirms a use of the present animal to study the underlying biochemistry of amyloid precursor protein (APP) or β-amyloid metabolism. Because of the expected increase in levels of β-amyloid with the 670/671 mutation, fewer copies of the mutated gene are needed . This special feature of the Swedish mutation addresses the generic concern about copy number and the increase chance of disruptive integration noted in some other cases.

What this means is that use of the claimed mutation facilitates the development of neuropathological β-amyloid plaques (and hence the Alzheimer's cascade) at lower overall expression levels of APP. Applicant disclosed the study of all of these parameters as utilities for the mutant APP mouse and, in fact, experiments have proven the truth of these statements. The Office is not justified in continuing to assert that the only recognized utility requires in every instance an Alzheimer's-specific neuropathology. For these and other reasons we have traversed the lack of enablement rejection and respectfully request the Office to withdraw the rejection.

Applicant provides a non-human transgenic animal in which a mutant APP can be expressed and wherein the biochemical pathways of APP metabolism that lead to the deposition, accumulation, and aggregation and deposition of β-amyloid can be studied. Specifically, the transgenic animal can be used as "a tool for defining the underlying biochemistry of APP and β-amyloid metabolism, which thereby provides a basis for rational drug therapy." See in the specification page 20, lines 24-26. Because the application teaches how to make a mouse expressing APPSwe, this is an enabled use of the claimed animal. Thus, the Office errs when it states that "it is the development of Alzheimer's related pathologies that ... is what is required for use in studying Alzheimer's disease progression and factors that affect progression or cause inhibition of Alzheimer's disease." See page 5, lines 17-20 in the Office Action. The Office also errs when it states that "[i]f the mice or animal only develops plaques, this is not sufficient to serve as a model as disclosed since plaques alone are not indicative of Alzheimer's disease." See page 6, lines 10-12 in the Office Action.

The Office has improperly imposed its own determination of a required use of the claimed invention in contravention of the uses taught in the application and in the art. Specifically, the Office has required that the claimed non-human transgenic animal must manifest senile plaques and neurofibrillary tangles for the animal to be useful for the study of the pathogenesis of AD. Applicant respectfully points out that the Office fails to understand the term "pathogenesis" when it incorrectly asserts that the only use for the claimed animal is to study the course of AD and its responses to, for example, drugs and/or environmental factors. "Pathogenesis" is defined as "[t]he development of a diseased or morbid condition." See Webster's II New College Dictionary, page 805 (Exhibit D). It is well understood that the initial biochemical steps that ultimately lead to a manifest disease constitute a part of the development of the disease. Thus, one skilled in the art would have recognized that an important use of the claimed invention is to study the development of AD via the earliest biochemical and physical changes in a brain that, over time, eventually manifests the further signs and symptoms of AD. An animal with abnormal APP processing, a hallmark of the Swedish mutation of the present invention, clearly has a use.

Furthermore, the Office has not based its case of lack of enablement on sufficient scientific evidence. "A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support." M.P.E.P. §2164.04. The Office has not met this burden.

It was known in the art, at the time of the filing of the application, that an abnormal aggregation of β-amyloid in the brain of a subject is the first step in the pathogenesis of AD. An attached reference (Exhibit E), "Amyloid deposition as the central event in the aetiology of Alzheimer's disease," Hardy et al., *Trends in Pharmacological Sciences*, 12(10):383-388, October 1991, states that "... a pathogenic mutation in the β-amyloid precursor protein (APP) gene on chromosome 21 suggests that APP mismetabolism and β-amyloid deposition are the primary events in the disease process. The occurrence of AD in Down syndrome is consistent with this hypothesis. The pathological cascade for the disease process is most likely to be: β-amyloid deposition → tau phosphorylation and tangle formation → neuronal death. The development of a biochemical understanding of this pathological cascade will facilitate rational design of drugs to intervene in this process." (See Abstract.) An abstract (attached as Exhibit F), "The seminal role of beta-amyloid in the pathogenesis of Alzheimer disease," Joachim et al., *Alzheimer Dis Assoc Disord*, 6(1):7-34, 1992, teaches that accelerated β-amyloid is an early and critical event in many patients with AD. Another example in the art at the time of filing of the instant application (attached as Exhibit G), "Deposits of amyloid beta protein in the central nervous system of transgenic mice," Wirak et al., *Science*, 253(5017):323-5, July 1991, teaches that "the 4-kilodalton human amyloid beta protein was expressed under the control of the promoter of the human amyloid precursor protein in two lines of transgenic mice. Amyloid beta protein accumulated in the dendrites of some but not all hippocampal neurons in 1-year-old transgenic mice. Aggregates of the amyloid beta protein formed amyloid-like fibrils that are similar in appearance to those in the brains of patients with Alzheimer's disease." (See

Abstract.) It was also established that abnormal APP processing results in β-amyloid aggregation.

Consistent with the knowledge in the art, the instant specification teaches several enabled uses for the claimed animal. For example, the claimed non-human transgenic animal "can be used to define further the underlying biochemical events involved in AD pathogenesis. Such models could be employed to screen for agents that alter the degenerative course of AD. For example, a model system of AD could be used to screen for environmental factors that induce or accelerate the pathogenesis of AD. In contradistinction, an experimental model could be used to screen for agents that inhibit, prevent, or reverse the progression of AD. Such models could be employed to develop pharmaceuticals that are effective in preventing, arresting, or reversing AD." See in the specification page 4, lines 25-34. Moreover, "such model systems provide a tool for defining the underlying biochemistry of APP and β-amyloid metabolism, which thereby provides a basis for rational drug design." See in the specification page 20, lines 23-26. Applicant is confident that the teaching in the specification, as pointed out above and to which the Examiner alludes, and the art from the time of filing show that this use was both explicitly stated and apparent from the art.

With regard to the Office's allegation that the present invention does not enable a mouse that can be used in a screening assay or otherwise as an Alzheimer's Disease model, applicant respectfully points out that the Office errs when it assumes that the mouse of the claimed invention must necessarily manifest β-amyloid in the brain to have a use as a model for Alzheimer's Disease. While the evidence described herein establishes that such a model can be produced without undue experimentation, in fact, the claimed transgenic mouse also has a valid enabled use in screening for compounds that affect amyloid precursor protein processing. For example, U.S. Patent No. 5,720,936, issued to Wadsworth et al. in 1998, provides a transgenic mouse comprising a DNA construct containing an APP gene operably linked to one of several promoters (e.g., NSE (as taught in the instant specification), human β actin gene promoter, human platelet derived growth factor B chain gene promoter, rat sodium channel gene promoter, mouse myelin basic protein gene promoter, human copper-zinc superoxide dismutase gene

promoter, and mammalian POU-domain regulatory gene promoter) that can be delivered to a fertilized mouse egg by microinjection techniques disclosed in the instant application. This transgenic mouse can be used to screen for compounds that may affect the production of APP and β-amyloid peptide in the animal. In this issued patent it is recognized by the Office that this use satisfies the requirements of section 112 because it is this screening method to which the issued claims are directed. It has been established that the claimed mouse can be made and that use in assays for compounds that can affect the amount of APP and β-amyloid peptide production in the animals is considered by the Office to be a use for section 112 purposes. The Office has clearly indicated that a transgenic animal can be used to study APP processing in the pathogenesis of AD without necessarily recapitulating the pathological cascade of AD. The Office's position on this point cannot be disregarded in the present case, where, as here, the issue has arisen in a different, but factually similar, case.

The Office cites previously recited references to allege the lack of enablement of the claimed invention. However, applicant respectfully asserts that these references are not relevant to the issue of enablement because the Office has arbitrarily determined its own use for the claimed invention. In fact, the references cited by the Office are, on balance, at least as supportive of the enablement of the claimed invention as they are supportive of the Office's assertion of non-enablement.

Specifically, the Office continues to reject applicant's arguments that prior disclosures of transgenic animals expressing APP isoforms would have led a person of skill to find the novel Swedish mutation interchangeable and to expect abnormal levels of β-amyloid production. The Office states that Hsiao, U.S. Patent No. 5,877,399, used the prion promoter to direct expression of the Swedish mutant DNA sequence and that this promoter was not contemplated in the instant specification. The Office also implies, without support, that it may be the specific promoter used that produced the phenotype seen. Not only is this implication unfounded, it is also not particularly relevant because art from the time of filing showed that the NSE promoter could drive APPSwe expression in a transgenic mouse. See discussion of Quon and Cordell below.

The Office cites Malherbe for the allegation that an NSE-APPSwe did not express sufficient levels to produce transgenic mice that produced β-amyloid, neuronal disorganization or reactive gliosis up to age one year. In Malherbe, the problem was not the promoter, as alleged by the Office. Rather, it was the design of the experiment that led to the reported lack of these disease indicators. More specifically, the mouse was examined after only one year. It is recognized in this art that significant deposition of β-amyloid often occurs after age one depending in part on expression level. The Hsiao mouse and the APP23 mouse show robust deposits at around that age although some show diffuse deposits from as early as six months – it is partly dependent on expression levels as well of course. (Hsiao, and *Proc. Natl. Acad. Sci. USA* Vol. 94, pp. 13287–13292, November 1997 STURCHLER-PIERRAT et al.) Thus, rather than showing that the Swedish mutation is not sufficiently expressed to provide a model animal, Malherbe at most shows that the construct with the NSE promoter takes longer than the chosen study period to manifest the deposits.

The Office dismisses applicant's assertion that Kappell was misapplied by the Office. Specifically, the Office states that Kappell sets forth reasoning why the production of transgenic animals is unpredictable, i.e., because there is no means to control where the transgene inserts into the genome of the animal, it is allegedly unpredictable as to whether the transgene will be silenced or deleted from the genome. The Office states that because the instant application offers no methodology to overcome the allegedly unpredictable site of transgene insertion, the invention is not enabled.

One of skill would know how to determine the proper site of transgene insertion through routine experimentation. The art regarding transgenic animals in general, and Swedish mutation mice specifically, showed that the problems suggested in some of the art could be routinely overcome. See, for example, Pierrat et al. and Quon, described above. The specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public. *In re Bucher*, 929 F.2d 660, 661 (Fed. Cir. 1991). The asserted fact basis for this rejection has been contradicted by numerous

examples from the same art. Thus, the rejection is believed to be unsupported, and its withdrawal is respectfully requested.

The Office dismisses applicant's assertion that Lannfelt was misapplied by the Office. Specifically, the Office states that Lannfelt does not state that the mutant APP isoforms will result in mice exhibiting Alzheimer's disease pathologies, only that they are more likely since they are associated with the disease.

As previously argued, the Office has failed to take the teaching of Lannfelt et al. as a whole, so that the conclusion drawn is a negative overstatement of Lannfelt et al.'s teaching. In fact, Lannfelt et al. supports the utility and enablement of the presently claimed animals by suggesting that the approach applicant teaches is more likely than other approaches to produce a useful animal. More specifically, Lannfelt et al. states that "... there are no published reports of the use of mutant AD pathogenic sequences in transgenics. It is likely that such sequences are more likely to lead to β-amyloid deposition. Unfortunately, this lack of reports almost certainly reflects the fact that most of the groups which have made such mice are either in commercial organizations, or sponsored by them." (Emphasis added.) See page 211, column 1, first full paragraph. Clearly, Lannfelt et al. implies that lack of reported successes is here attributed to the lack of reporting, not the lack of successes.

Further, Lannfelt et al. supports applicant's assertion that the claimed invention is useful, specifically as a model for studying APP metabolism and β-amyloid aggregation. Lannfelt et al. teaches that "β-amyloid deposition in the brain is a diagnostic hallmark of AD, and has most probably a causative role in the disease. β-amyloid is a highly aggregating ~ 40 amino acid peptide that is a break down product of the larger precursor, APP." See page 208, col. 1, paragraph 1. An animal having a hallmark of AD has as a use that is the study of the hallmark and its development.

Thus, rather than being relevant for teaching that the production of the present mutant APP mouse model would be problematic, Lannfelt et al. is highly relevant in its suggestions that mutant APP-expressing mice as claimed are more likely to be useful and that some of these transgenic mice had already been made. Further, Lannfelt et al. teaches that β-amyloid is

important in the pathogenesis of AD and that studying its metabolism is useful for learning about the development of AD. This is a use that is specifically taught in the present application.

The Office Action rejects applicant's argument that the Office has misapplied Higgins. Specifically, the Office alleges that because mice with the APP695 mutation rarely manifested immunoreactive deposits that stain with mAb 4.1, the claimed invention is unpredictable.

"The 'predictability or lack thereof' in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is predictability in the art." M.P.E.P. § 2164.03. The art can admit some degree of unpredictability and still support enablement by showing that one of skill views the potential problems as an accepted aspect of the experimentation in which one would routinely engage. Furthermore, there is little question in the art whether a mouse with the Swedish mutation will predictably produce abnormal levels of β-amyloid, which in itself is a use.

More specifically, the Office's assertion regarding the teaching of Higgins et al. is not supported in that reference. In fact, Higgins et al. teaches that mice can express the human APP 695 gene and produce β-amyloid peptide, although this is rare. Moreover, what the Office fails to acknowledge is that Higgins et al. shows that transgenic mice that express the human APP 751 gene did indeed manifest neuropathologies associated with Alzheimer's Disease. Specifically, mice expressing the human APP 751 gene had preamyloid deposits that stained with a monoclonal antibody (mAb 4.1). (See page 225.) Further, the APP 751 mice showed evidence of numerous neuropil threads and neurofibrillary tangles, detected with monoclonal antibody Alz50. These features are described as "features of early Alzheimer's disease (AD) pathology." (See Abstract). Thus, Higgins et al. supports the enablement of the claimed non-human transgenic animal, even though some unpredictability may exist.

The Office continues to allege that mice as a species are resistant to expressing a mutant APP gene, for example APP695, as stated in Higgins et al. and rejects applicant's argument that Hsiao ('399) shows that mice expressing APP695 have AD pathologies. The Office states that because Hsiao used a prion promoter with the mutated nucleotide sequence and obtained AD

pathologies, and because Malherbe showed that mice with NSE-APP695Swe did not develop β-amyloid deposits, that it is reasonable to conclude that the prion promoter is the agent that brought the particular Alzheimer's pathologies seen in the mice in Hsiao.

"In order to make a rejection, the Examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention." *In re Wright*, 999 F.2d 1557 (Fed Cir. 1993). (Underline added.) "A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. Assuming that sufficient reason for such doubt exists, a rejection for failure to teach how to make and/or use will be proper on that basis. *In re Marzocchi*, 439 F.2d 220, 224 (CCPA 1971). As stated by the court, 'it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement." (Underline added.) To the extent that the Office asserts the 695 isoform is resistant to plaque formation, this is irrelevant to the other asserted utilities in mice still expressing the isoform. To the extent that the Office may, regardless, continue to rely upon Higgins and Malherbe for the assertion, applicant has rebutted the Office assertion by an evidentiary showing that the overall expression level is responsible for plaque formation.

The Office's allegation that "it is reasonable to conclude that the prion promoter is the agent that brought the particular Alzheimer's pathologies observed in the Hsiao mice" is not supported by any acceptable scientific evidence. In fact, the Office's statement is *contra* to the knowledge in the art at the time of filing of the present application. For this reason, applicant believes that this rejection has no basis and respectfully requests its withdrawal.

One skilled in the art would recognize at the time of filing of the instant application that the level of transcription of a mutant nucleotide sequence could be affected by the choice of promoter. Thus, it would be routine for a person of skill to construct various expression vectors with different promoters to look for a transgenic animal with increased expression of mutant APP and β-amyloid. Further, applicant believes it is error for the Office to assume that the prion promoter of Hsiao was responsible for the amyloid deposits seen in those mice. Instead, the promoter produced a level of expression of APP that was conducive to the formation of the β-amyloid deposits. Thus, applicant believes that this basis for the rejection should be withdrawn.

The Office Action rejects applicant's argument that the Office has misapplied Felsenstein. Specifically, the Office alleges that the claimed invention is not enabled because Felsenstein states that no animal model exists that can recapitulate the pathological cascade of Alzheimer's Disease. The Office also alleges that, even though Felsenstein acknowledged that rats may not be the proper model for AD and that older rats, had they been studied, may have manifested β-amyloid deposits, because the instant application does not teach that older rats would have such deposits, the claimed invention is not enabled. The Office further states that as there is no disclosure in the instant specification for the use of the animals to study APP processing, applicant cannot at this point say that is their invention.

The rats in Felsenstein are exemplary of only one species of animal, and one failure to demonstrate lack of APP expression in rats does not mean that the claimed invention is not enabled. Moreover, applicant believes that the rats were too young when they were studied so that the reported results do not necessarily reflect what would have resulted, had the experiment been carried out for a longer period of time. Further, as asserted above, the instant application does disclose use of the claimed non-human transgenic animal for studying APP processing and biochemical pathways.

The Office's failure to address applicant's argument regarding Quon and U.S. Patent No. 5,387,742, issued to Cordell is significant. The Quon mouse is a credible model for studying the neuropathology of AD. Quon et al. supports the enablement of a non-human transgenic animal that expresses a mutant APP driven by the NSE promoter, and the reference also supports the

usefulness of an animal model to study the pathogenesis, i.e., development, of the neuropathological findings of AD without requiring that the animal have late manifestations of AD. This reference was published and known to persons of skill in the art about one year before the filing of the instant application. These authors constructed a chimeric gene comprising human APP 751 and rat neural-specific enolase (NSE) and expressed the construct in mice, resulting in the deposition of β-amyloid deposits in the cortex and hippocampal brain regions of the transgenic mice that distinguished the transgenic mice from control mice. Thus, at the time of filing of the present application, it was known in the art how to make and use a transgenic mouse that expresses a human APP. The present application teaches a construct comprising a mutant APP770 and a neural-specific enolase (NSE) promoter. Because the steps for making these animals are essentially the same, regardless of the APP isoform, a transgenic animal containing the NSE promoter and a mutant APP 770 gene, as enabled in the application, could be made without undue experimentation and reasonably expected to have a substantial and specific use.

Further evidence of the state of the art of making transgenic animals at the time the instant application was filed, is found in U.S. Patent No. 5,387,742, which was filed on June 17, 1991 (about one year before the filing date of the instant application) and issued to Cordell et al. in 1995. In the '742 patent, the patentee teaches how to make and use two strains of transgenic mice, one strain whose cells contain a DNA sequence, comprising a nerve tissue specific promoter (rat NSE) operably linked to a DNA sequence that encodes APP 751, and the other strain whose cells contain a DNA sequence, comprising rat NSE operably linked to a DNA sequence that encodes APP 770. Each DNA construct is integrated in the genome of the mice and expressed to form β-amyloid deposits in the brain of the mice. See col. 36, lines 25-36. The '742 patent is evidence that at least one year before the instant application was filed, persons of skill in the art had the knowledge to make and use transgenic mice that express an APP sequence that encodes APP and forms β-amyloid deposits in the brain of the mice without undue experimentation.

Because the teaching of the '742 patent is presumed enabled, applicant believes that the facts of the instant application, i.e., the disclosure of a non-human transgenic animal that can express a mutant APP, when assessed against the same legal standard, as applied to similar facts in the '742 patent, should lead the Office to come to the same conclusion. Specifically, the claimed invention is enabled.

In conclusion, applicant believes that, given the knowledge of the art and the teachings of the instant application, one of skill, at the time of filing of the application, would have been able to make the claimed non-human transgenic animal. Moreover, one skilled in the art could use the claimed invention to study the underlying biochemistry of APP and β-amyloid metabolism, which thereby provides a basis for rational drug design. Because these uses were disclosed in the application and validated in the art at the time of filing, applicant believes that the claimed invention is enabled and respectfully requests that this rejection be withdrawn.

Pursuant to the above amendments and remarks, reconsideration and allowance of the pending application are believed to be warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

Credit Card Payment Form PTO-2038 authorizing payment in the amount of $895.00 (representing $375.00 RCE fee; $465.00 three-month extension of time fee; and $55.00 Terminal Disclaimer fee—SMALL ENTITY), is enclosed. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required or to credit any overpayment to Deposit Account No. 14-0629.

Respectfully submitted,

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27th Edition

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Medical Dictionary

W.B. SAUNDERS COMPANY
A Division of Harcourt Brace & Company
Philadelphia London Toronto
Montreal Sydney Tokyo

Dorland's illustrated medical dictionary.
Philadelphia: W.B. Saunders Co.,
v.: ill.; 27 cm.
Irregular.
Began publication with 23rd ed.
Description based on: 26th ed.
Continues: American illustrated medical dictionary.

1. Medicine—Dictionaries. I. Dorland, W.A. Newman (William Alexander Newman), 1864–1956.
[DNLM: 1. Dictionaries, Medical. 2. Reference Books, Medical]

R121.D73 610'.3'21—dc19 0-638
AACR 2 MARC-S

Library of Congress [8607r85]rev6

W.B. SAUNDERS COMPANY
A Division of
Harcourt Brace & Company

The Curtis Center
Independence Square West
Philadelphia, PA 19106

Listed here are the latest translated editions of this book together with the languages for the translations and the publishers.

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Japanese *(26th Edition)*—Hirokawa Publishing Company, Tokyo, Japan

Spanish *(26th Edition)* (Adaption)—Nueva Editorial Interamericana, Mexico City, Mexico

Managing Editor, Dictionaries: Elizabeth J. Taylor
Editors: Douglas M. Anderson, Joseph M. Patwell, Katharine Plaut, Kathleen McCullough
Production Manager: Carolyn Naylor
Illustrator: Sharon Iwanczuk
Illustration Coordinator: Walt Verbitski
Mechanical Artist: Melissa Walter

Dorland's Illustrated Medical Dictionary ISBN 0-7216-3154-1

Library of Congress catalog card number 78-50050

Last digit is the print number: 9 8 7 6

mnemic (ne′mik) mnemonic.

mnemonic (ne-mon′ik) [Gr. *mnēmonikos* pertaining to memory] pertaining to, characterized by, or promoting recollection, or memory.

mnemonics (ne-mon′iks) the cultivation or improvement of memory by special methods or techniques.

M.O. Medical Officer.

Mo chemical symbol for *molybdenum*.

Moban (mo′ban) trademark for a preparation of molindone hydrochloride.

Mobilina (mo″bĭ-li′nah) [L. *mobilis* mobile] a suborder of mobile, usually conical or cylindrical or discoidal and orally and aborally flattened ciliate protozoa (order Peritrichida, subclass Peritrichia), characterized by the presence of a ciliary girdle and a complex thigmotactic apparatus at the aboral end, often with a highly distinctive denticulate ring of "teeth." All species are ectoparasites or endoparasites of freshwater or marine vertebrates and invertebrates, and those found on the gills of fish are pathogenic.

mobility (mo-bil′ĭ-te) [L. *mobilitas*] 1. capability of movement, of being moved, or of flowing freely. 2. rate of movement of a charged particle in an applied electric field. **electrophoretic m.,** 1. the rate of migration (in cm/s) per unit electric field strength (V/cm) of a charged particle in electrophoresis. Symbol, μ. 2. any measure of the rate of migration of an ionic species in electrophoresis, e.g., β electrophoretic mobility, designating the electrophoretic mobility of a beta globulin.

mobilization (mo″bĭ-li-za′shun) the process of making a fixed part or stored substance mobile, as by separating a part from surrounding structures to make it accessible for an operative procedure or by causing release into the circulation for body use of a substance stored in the body. **stapes m.,** surgical correction of immobility of the stapes, in treatment of deafness resulting from otosclerosis.

mobilometer (mo″bil-om′ĕ-ter) an instrument for measuring the consistency of liquids such as oil, cream, liquid foods, etc.

Möbius' disease, sign, syndrome (me′be-us) [Paul Julius *Möbius*, German neurologist, 1853–1907] see under *disease, sign,* and *syndrome*.

MOCA a regimen of methotrexate, Oncovin (vincristine), cyclophosphamide, and Adriamycin (doxorubicin), used in cancer chemotherapy.

moccasin (mok′ah-sin) a common name applied to several species of snakes, but usually denoting the venomous semiaquatic pit viper *Agkistrodon piscivorus*, or water moccasin, less frequently *A. contortrix*, or Highland moccasin. See table accompanying *snake*.

mocezuelo (mo″se-zwa′lo) [Mexican] trismus neonatorum.

mock-up (mok′up) a full-sized model of an apparatus or other equipment constructed out of substitute materials, used in instruction or for study and improvement of design.

modality (mo-dal′ĭ-te) 1. a homeopathic term signifying a condition which modifies drug action; a condition under which symptoms develop, becoming better or worse. 2. a method of application of, or the employment of, any therapeutic agent; limited usually to physical agents. 3. a specific sensory entity, such as taste.

mode (mōd) [L. *modus* measure, manner] 1. the value at which the peak of the theoretical frequency distribution underlying the sample data occurs; usually referred to as the most frequently occurring value in a statistical sample or population. 2. a relative maximum of the density function of a probability distribution, a value occurring more frequently than values immediately above and below; a distribution with two peaks is bimodal.

model (mod′el) 1. something that represents or simulates something else; a replica. 2. a reasonable facsimile of the body or any of its parts; used for demonstration and teaching purposes. 3. cast, def. 5. 4. to imitate another's behavior; see *modeling*. **animal m.,** any condition found in an animal that is of value in studying a biological phenomenon, e.g., a pathological mechanism of an animal disorder useful in studying human disease.

modeling (mod′el-ing) a behavior modification technique in which the patient is taught to imitate the desired behavior of another.

moderator (mod′er-a-tor) in nuclear chemistry and physics, a substance, such as graphite or beryllium, used to cut down the flux of subatomic particles or radiation by absorption of the same.

Moderil (mod′er-il) trademark for a preparation of rescinnamine.

modification (mod″ĭ-fi-ka′shun) the process or result of changing the form or characteristics of an object or substance. **behavior m.,** see under *therapy*. **racemic m.,** see *racemate*.

modioliform (mo″de-o′lĭ-form) shaped like the hub of a wheel.

modiolus (mo-di′o-lus) [L. "nave," "hub"] [NA] the central pillar or columella of the cochlea; called also *columella cochleae*.

Mod. praesc. abbreviation for L. *mo′do praescrip′to*, in the way directed.

modulation (mod″u-la′shun) [L. *modulare* to measure] the normal capacity of cell adaptability to its environment. **antigenic m.,** alteration or loss of reactivity of cell surface antigens resulting from redistribution of antigenic sites due to the presence of bound antibody.

modulator (mod″u-la′tor) a specific inductor that brings out characteristics peculiar to a definite region.

Moduretic (mod″u-ret′ik) trademark for preparations of amiloride hydrochloride with hydrochlorothiazide.

MODY maturity-onset diabetes of youth.

Moe plate (mo) [John H. *Moe*, American surgeon, born 1905] see under *plate*.

Moebius see *Möbius*.

Moeller's glossitis (me′lerz) [Julius Otto Ludwig *Moeller*, German surgeon, 1819–1887] see under *glossitis*.

Moeller-Barlow disease (me′ler-bar′lo) [J. O. L. *Moeller*; Sir Thomas *Barlow*, London physician, 1845–1945] see under *disease*.

Moenckeberg (menk′ĕ-berg) see *Mönckeberg*.

Moentjang tina the Malay term in Indonesia for intoxication caused by the use in food of oil obtained from the fruit of the tropical tree *Hernandia sonora* L. (Hernandiaceae). Ordinarily the oil is used only in lamps.

Moerner-Sjöqvist method, test (mer′ner-syek′vist) [Carl Thore *Moerner*, Swedish physician, 1864–1917; John August *Sjöqvist*, Swedish physician, 1863–1934] see *Sjöqvist method*, under *method*.

mogi- [Gr. *mogis* with difficulty] a combining form meaning difficult, or with difficulty.

mogiarthria (moj-e-ar′thre-ah) [*mogi-* + Gr. *arthroun* to utter distinctly + *-ia*] a form of dysarthria in which there is defective coordination of the muscles involved.

mogilalia (moj-e-la′le-ah) [*mogi-* + *lalia* chatter] difficulty in speech; stuttering.

mogiphonia (moj-e-fo′ne-ah) [*mogi-* + Gr. *phōnē* voice] difficulty in making vocal sounds.

Mohr's test (mōrz) [Francis *Mohr*, American pharmaceutical chemist] see under *tests*.

Mohrenheim's fossa, triangle (mo′ren-hīmz) [Baron Joseph Jacob Freiherr von *Mohrenheim*, Austrian surgeon, 1759–1799] fossa infraclavicularis.

Mohs hardness number (mōz) [Friedrich *Mohs*, German mineralogist, 1773–1839] see under *number*.

Mohs' technique (chemosurgery, surgery) (mōz) [Frederic Edward *Mohs*, American surgeon, born 1910] see under *technique*.

moiety (moi′ĕ-te) [Fr. *moitié*, from L. *medietas, medius*, middle] any equal part; a half; also any part or portion. **carbohydrate m.,** a carbohydrate-derived portion of the structure of a molecule. **corrin m.,** a complex ring system in the vitamin B_{12} molecule, closely related to the porphyrins of the cytochromes.

moist (moist) somewhat wet; damp.

mol (mol) mole, def. 3.

molal (mo′lal) containing one mole of solute per kilogram of solvent. NOTE: *molal* refers to the weight of the solvent, *molar* to the volume of the solution.

molality (mo-lal′ĭ-te) the number of moles of a solute per kilogram of pure solvent. NOTE: *molality* refers to the weight of the solvent, *molarity* to the volume of the solution.

molar (mo′lar) 1. [L. *moles* mass] pertaining to a mass; not

Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 13287–13292, November 1997
Neurobiology

Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology

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Communicated by Floyd E. Bloom, The Scripps Research Institute, La Jolla, CA, September 15, 1997 (received for review July 21, 1997)

ABSTRACT **Mutations in the amyloid precursor protein (APP) gene cause early-onset familial Alzheimer disease (AD) by affecting the formation of the amyloid β (Aβ) peptide, the major constituent of AD plaques. We expressed human APP_{751} containing these mutations in the brains of transgenic mice. Two transgenic mouse lines develop pathological features reminiscent of AD. The degree of pathology depends on expression levels and specific mutations. A 2-fold overexpression of human APP with the Swedish double mutation at positions 670/671 combined with the V717I mutation causes Aβ deposition in neocortex and hippocampus of 18-month-old transgenic mice. The deposits are mostly of the diffuse type; however, some congophilic plaques can be detected. In mice with 7-fold overexpression of human APP harboring the Swedish mutation alone, typical plaques appear at 6 months, which increase with age and are Congo Red-positive at first detection. These congophilic plaques are accompanied by neuritic changes and dystrophic cholinergic fibers. Furthermore, inflammatory processes indicated by a massive glial reaction are apparent. Most notably, plaques are immunoreactive for hyperphosphorylated tau, reminiscent of early tau pathology. The immunoreactivity is exclusively found in congophilic senile plaques of both lines. In the higher expressing line, elevated tau phosphorylation can be demonstrated biochemically in 6-month-old animals and increases with age. These mice resemble major features of AD pathology and suggest a central role of Aβ in the pathogenesis of the disease.**

The pathological hallmarks of Alzheimer disease (AD) are amyloid plaques and neurofibrillary tangles (1) composed of amyloid β (Aβ), a 39- to 43-amino acid peptide (2, 3) and hyperphosphorylated tau (4, 5). In addition, extensive neuritic degeneration has been described including a dramatic loss of cholinergic fibers (6, 7). The Aβ peptide is thought to play a central role in AD pathogenesis, as suggested by mutations in the gene encoding its precursor, amyloid precursor protein (APP) that are linked to forms of early-onset familial AD (FAD) (8, 9). These mutations either cause an elevated production of total Aβ (10, 11) or increase the more fibrillogenic Aβ-(1–42) (12, 13).

Numerous attempts focusing on transgenic expression of human APP (14–19) have been made to obtain a valid animal model of AD. However, only two transgenic mouse lines have been described that develop Aβ deposits characteristic of AD (20, 21). We have generated various transgenic mice that express human APP_{751}. Two of these lines that differ in transgene-derived APP expression levels and FAD mutations develop plaque-like Aβ deposits in neocortex and hippocampus to different degrees. They also display additional aspects of AD pathology not commonly associated with the Aβ peptide.

MATERIALS AND METHODS

Expression Constructs and Transgenic Mice. Transgenic constructs used in this study contain human (19) or murine (22) Thy-1 expression cassettes and human APP_{751} cDNAs starting with an optimized Kozak consensus sequence and extending to nucleotide 3,026 (*Hin*dIII site) as described (19). The human APP cDNAs carry either the Swedish double mutation at positions 670/671 (KM->NL) (8) alone or in conjunction with the London mutation (V717I) (9). Construct APP 14 utilizes the human Thy-1 cassette to drive human APP_{751} Swedish and was described in detail in a previous study (19). The APP 22 expression construct is identical to APP 14 except for the additional V717I mutation in the human APP cDNA. It was generated by replacing a 600-bp *Bgl*II–*Spe*I fragment of human APP_{751} cDNA with the corresponding fragment carrying the V717I mutation. To generate the construct for APP 23, the APP_{751} cDNA with the Swedish mutation was inserted into the blunt-ended *Xho*I site of the expression cassette containing the murine Thy-1.2 gene (22). Vector sequences were removed by *Not*I and *Not*I/*Pvu*I digestion for APP 14/APP 22 and APP 23, respectively. Injection and manipulation of mice were identical to described procedures (19).

RNA Quantification. Transgene mRNA was quantified by reverse transcription-coupled PCR (23). In brief, total RNA from brains of transgenic mouse lines APP 14, 22, and 23 was isolated and transcribed into cDNA. Fragments of APP harboring the Aβ region were amplified by PCR using primers 5′-ACCACCGTGGAGCTCCTTCCCGTGAA-3′ and 5′-GCAACTGCAGTGTGTACTGTTTCTTC-3′, specific for sequences common in human and murine APP. Fragments were directionally subcloned into M13 replicative form DNA, and dual filter lifts from plaque-containing plates were hybridized with oligonucleotides 5′-AATTCTGCATCCATCTTCACTTCCGA-3′ and 5′-AATTCTGCATCCAGATTCACTTCAGA-3′, discriminating mouse wild-type and mutated human APP.

***In Situ* Hybridization.** *In situ* hybridization was performed as described (24). A ^{33}P-labeled oligonucleotide probe, 5′-AGCCTCTTCCTCTACCTCATCACCATCCTCATCGTCCTC-

Abbreviations: Aβ, amyloid β; AD, Alzheimer disease; APP, amyloid precursor protein; FAD, familial AD; MHC, major histocompatibility complex; GFAP, glial fibrillary acidic protein.

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G-3′, complementary to coding sequence of human APP between nucleotides 859 and 898 was used at a final concentration of 2 pmol/ml. The hybridized slides were exposed to β-max film (Amersham) for 3 days.

Western Blots. Cerebral cortices were homogenized in 10 vol of 50 mM Tris·HCl, pH 8/1% Nonidet P-40/5 mM dithiothreitol/100 mM NaCl/5 mM EGTA/50 mM NaF/1 mM sodium orthovanadate/100 nM ocadaic acid/1× complete protease inhibitor mixture (Boehringer Mannheim) mixed with an equal volume of 2× SDS sample buffer and processed as described (25). Samples were electrophoresed on SDS/polyacrylamide minigels, the proteins were transferred to Immobilon P (Millipore) membranes, and the Western blots were probed with antibodies AT8 (Innogenetics, Gent, Belgium) (26, 27), R27 (28) and tau7, which was raised against tau purified from adult rat brain and recognizes tau from multiple species (29). Peroxidase-conjugated goat anti-mouse IgG (Dianova, Hamburg, F.R.G.) or peroxidase-coupled goat anti-rabbit IgG (Dianova) was used as a second antibody. The immunoblots were developed with the ECL system (Amersham).

Histology. Heterozygous mice from line APP 22 were analyzed at 12 (n = 3) and 18 months (n = 3). Heterozygous APP 23 mice were sacrificed at 6 (n = 9), 9 (n = 12), 12 (n = 6), 15 (n = 4), 18 (n = 3), and 24 (n = 3) months. Equivalent numbers of nontransgenic littermates served as controls. Mice were perfused with 4% paraformaldehyde in phosphate-buffered saline. Paraffin sections (4 μm) of mouse brain were stained with hematoxilin/eosin, methenamine silver, Congo Red, Holmes luxol, and Gallyas silver iodide (30). Immunohistochemical staining was performed on deparaffinized brain sections. Anti-MAC-1, acetylcholinesterase (31), major histocompatibility complex (MHC) class II, complement C3, and anti-tau histochemistry was performed with 40-μm free-floating fixed frozen sections. To enhance antigenicity for NF200, glial fibrillary acidic protein (GFAP), and tau antibodies, dewaxed sections were rehydrated and treated for 30 min by immersion in citrate buffer at 90°C. Primary antibodies directed against the Aβ peptide (32) are summarized in Table 1. In addition, anti-heparan sulfate monoclonal antibody 10E4 (Seikagu, Tokyo); anti-neurofilament NF200 (NovoCastra, Newcastle, U.K.); anti-APP 474 raised against purified rat APP_s; anti-apolipoprotein E antibody; anti-MAC-1 and anti-MHC class II (Serotec); anti-GFAP and anti-phosphotyrosine PT-66 (Sigma); anti-mouse complement C3 (Cappel, ICN); anti-PHF1 (33) recognizing tau phosphoserine 396 and 402; AT8 monoclonal antibody (Innogenetics) (26, 27) recognizing phosphoserine 202; R27 (28) raised against phosphorylated serine 396; R32 (28) raised against phosphorylated serine 262, N-tau 5 raised against a tau peptide as described (34), and monoclonal antibody Alz50 (35) were used. Sections were treated with normal serum to block nonspecific sites and incubated overnight at 4°C with the primary antibody. Bound antibody was visualized by using a Vectastain ABC Elite kit (Vector Laboratories).

Table 1. Aβ antibodies recognizing mouse and human plaques

Antibody	Antigen residues
Dako	8–17
NT11	1–40
β1	1–40
AS 1–5*	1–5
AS 12–28	12–28
AS 40/18*	17–40
AS 42/14*	36–42
AS 43/15*	36–43

*These antibodies specifically recognize Aβ amino acids 1, 40, 42, and 43, respectively.

RESULTS

APP Overexpression in Brains of Transgenic Mouse Lines Controlled by the Thy-1 Promoter. Expression cassettes containing the Thy-1 promoter were used to drive neuron-specific APP expression in transgenic mice (Fig. 1). The two mouse lines APP 14 (19) and APP 22 carry the human Thy-1 promoter to drive expression from different APP cDNAs. The APP 14 construct contains human APP_{751} with the Swedish double mutation at positions 670/671 (KM → NL), the APP 22 expression unit carries the London mutation (V717I) in addition. Both constructs result in comparable spatial expression patterns with highest transgene-derived mRNA levels in neocortex and hippocampus as shown for APP 22 mice (Fig. 2*A*). In both lines, mRNA levels exceed endogenous APP mRNA by 2-fold (data not shown) as determined by semi-quantitative PCR. In a third line, APP 23, APP_{751} Swedish expression is driven by the murine Thy-1 promoter. The spatial expression pattern (Fig. 2*B*) is qualitatively similar to APP 14 and APP 22 mice; however, the transgene is 7-fold overexpressed. The degree of APP overexpression was confirmed by Western blotting for all three mouse lines (data not shown).

APP 22 and APP 23 Transgenic Mice Develop Aβ Deposits in Neocortex and Hippocampus. Whereas APP 14 mice do not show any indication of AD pathology up to an age of 2 years (data not shown), Aβ deposits are detected in neocortex and hippocampus of 18-month-old but not 12-month-old APP 22 mice (Fig. 2*C*). The higher expressing APP 23 line shows first rare deposits at 6 months of age. They increase with age in size and number and eventually occupy a substantial area of the neocortex and hippocampus in 24-month-old mice (Fig. 2*D*). At this age deposits are also found in thalamus and olfactory nucleus and isolated in the caudate putamen. In both lines, all deposits show immunoreactivity with different antibodies specific for Aβ (Fig. 2 *C* and *D* and Table 1), which are comparable to the results obtained on brains from AD patients (data not shown). It is noteworthy that immunoreactivity with the end-specific Aβ42 antibody in APP 22 mice appears similar in intensity to that obtained in sections from the higher-expressing APP 23 mice, reflecting the impact of the mutation at position 717 on the production of the more amyloidogenic Aβ-(1–42) (12). Typical plaque-associated proteins in AD such as heparan sulfate proteoglycan and apolipoprotein E, which bind to Aβ, are also present in most deposits (data not shown). No differences are seen between male and female mice.

Differences in Plaque Anatomy in APP 22 and APP 23 Mice. When compared directly, the intensity of the Aβ and heparan sulfate proteoglycan-specific immunostaining is much stronger

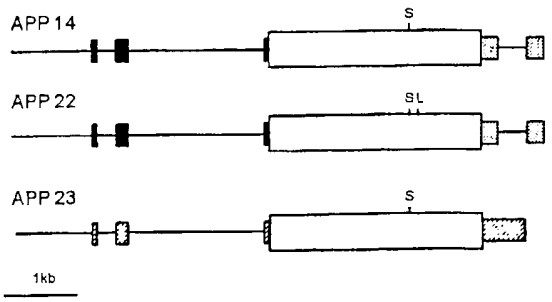

FIG. 1. Transcription units for APP transgenic mice. The diagrams are linear representations of the injected expression constructs and are labeled accordingly. Exons of the human Thy-1 gene are illustrated as solid boxes, and stippled boxes indicate sequences of simian virus 40 tumor antigen at the 3′ end of APP 14 and APP 22 constructs. Hatched boxes represent the murine Thy-1 exons used in the APP 23 construct. APP_{751} cDNAs are shown as open boxes with vertical lines marking the positions of the respective Swedish (S) and London (L) FAD mutations.

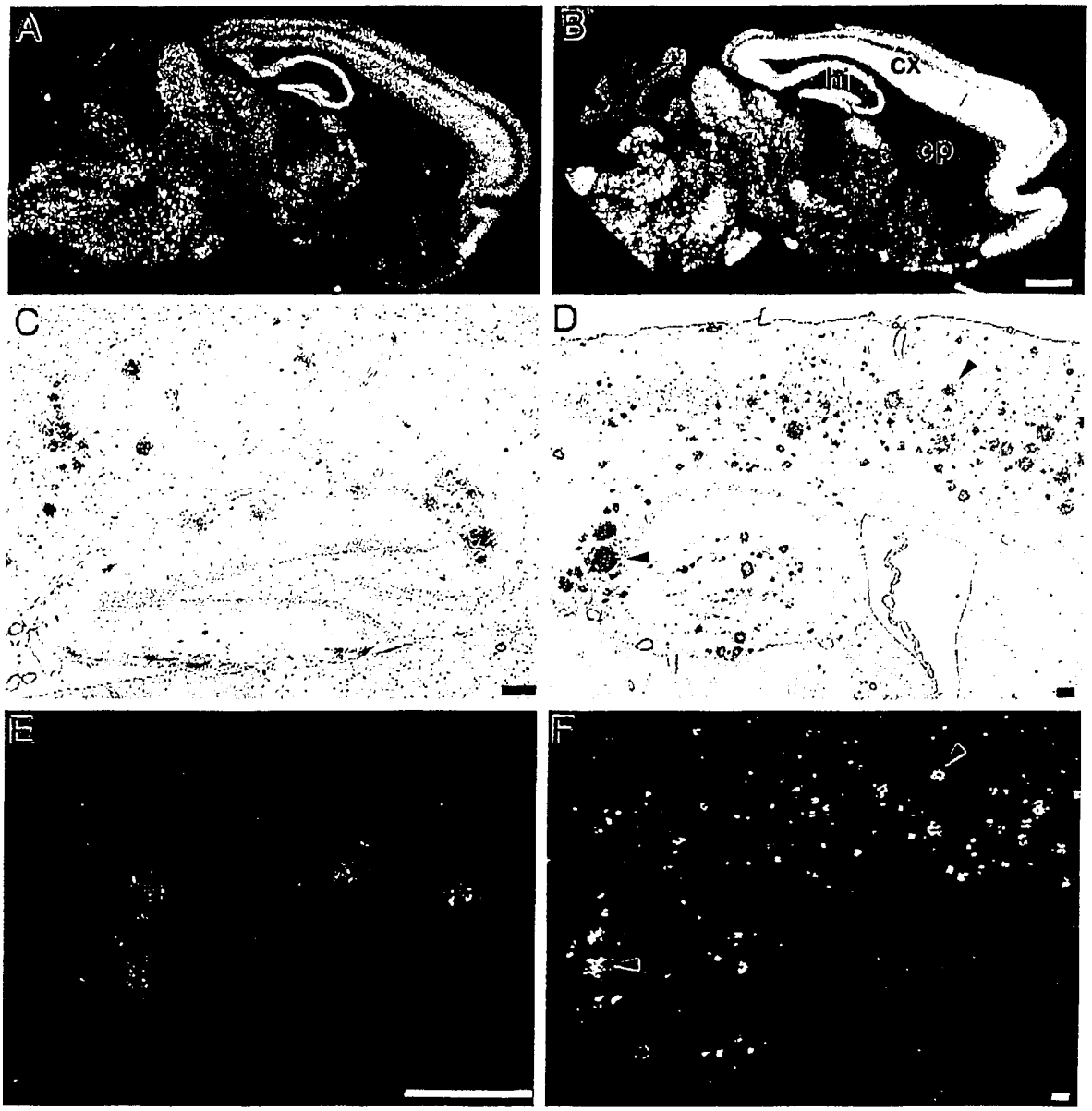

FIG. 2. Expression of APP mRNA and deposition of Aβ in brains of transgenic mice. (*A*, *C*, and *E*) Sections taken from 18-month-old APP 22 mice. (*B*, *D*, and *F*) Sections from 24-month-old APP 23 mice. *In situ* hybridization on sagittal sections of brains from mouse line APP 22 (*A*) and APP 23 (*B*) was performed with an oligonucleotide probe specific for human APP. cp, Caudate putamen; cx, cortex; hi, hippocampus. (Bars = 1 mm.) Immunohistochemistry (*C* and *D*) and Congo Red staining (*E* and *F*) of sagittal sections from brains of transgenic mice. Immunostaining was performed with an antibody raised against Aβ8–17 (Dako). (Bars = 100 μm.) Arrowheads point to identical deposits in *D* and *F*.

in deposits of APP 23 mice. Consistent with this notion, the majority of deposits in APP 22 mice are of the "diffuse" type. Relatively few Congo Red-positive plaques are present in the neocortex and the subiculum (Fig. 2*E*). In contrast, almost all extracellular deposits in APP 23 mice are intensely stained with methenamine silver and at their first appearance already show Congo Red birefringence (Fig. 2*F*) indicative of dense core plaques.

Inflammatory Responses in Brains of APP Transgenic Mice. In addition to Aβ deposition, inflammatory processes reminiscent of AD occur in these mice. In both lines, a massive glial response can be demonstrated in brain areas with plaques, which is more pronounced in APP 23 mice and hence correlates with the higher burden of congophilic plaques. Immunocytochemistry for GFAP (Fig. 3*A*), phosphotyrosine (Fig. 3*C*), MAC-1 (Fig. 3*B*), MHC class II, and complement C3 suggest a contribution from both astrocytes and microglia.

Neuritic Loss and Distortion of Cholinergic Fibers Are Observed in the Vicinity of Plaques. Plaques are surrounded by dystrophic neurites visualized by neurofilament (Fig. 4*C*), APP, and synaptophysin (data not shown) immunostaining that are only seen at the outer margin of dense compacted Aβ deposits. In areas of high cell density, such as hippocampal pyramidal cells, a reduction of cell bodies adjacent to the Aβ peptide deposition is apparent (Fig. 4*D*). Although this may simply be cell displacement, the lack of compression of the intracellular space suggests local loss of neurons in the plaque vicinity. When stained for acetylcholinesterase, a strong labeling of plaques and a local distortion of the cholinergic fiber network is observed (Fig. 4*A*). Acetylcholinesterase activity is

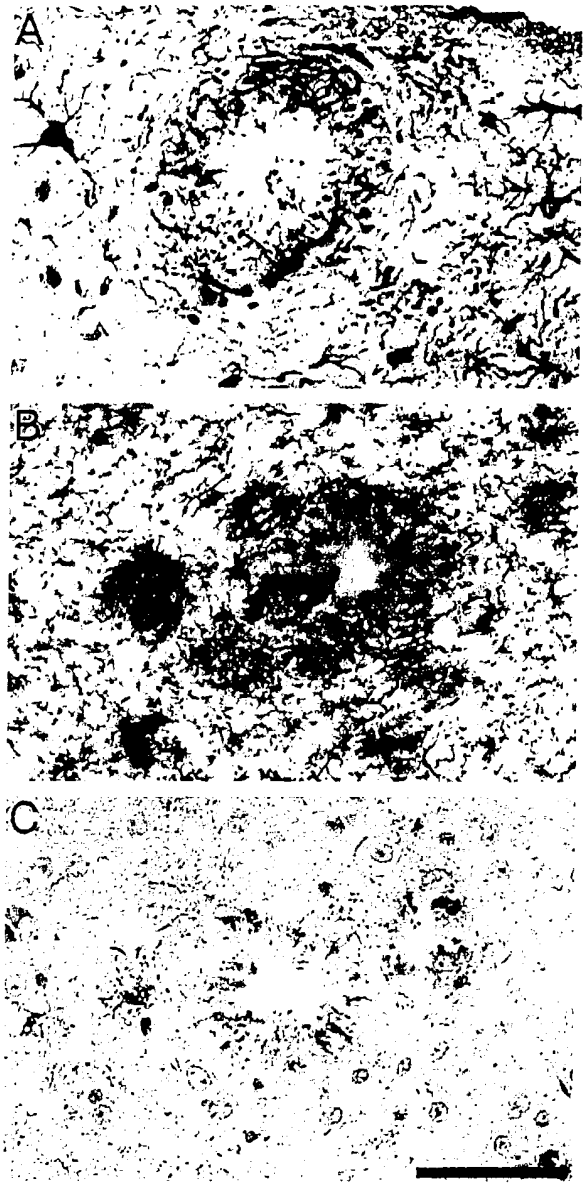

FIG. 3. Characterization of inflammatory processes in 12-month-old APP 23 transgenic mice. Immunostaining with a GFAP-specific antibody (*A*) indicates hypertrophic astrocytes. Activated microglia are visualized by immunostaining with a MAC-1 antibody (*B*) and a phosphotyrosine antibody (*C*). Both glia cell types are intimately associated with Aβ deposits. (Bar = 50 μm.)

found throughout the amyloid plaques and in enlarged neurites at the plaque periphery (Fig. 4*A*).

Congophilic Plaques Are Surrounded by Distorted Neurites Containing Hyperphosphorylated Tau. When examined for aspects of neurofibrillary pathology, hyperphosphorylated microtubule-associated protein tau can be detected in transgenic mouse brains of both lines. Immunostaining with the AT8 antibody is exclusively associated with Congo Red-positive plaques in APP 22 and APP 23 mice. It highlights structures resembling distorted neurites surrounding the core of the deposits (Fig. 5 *A* and *B*). Similar reactions are obtained with the antibodies PHF1, R27, and R32 (data not shown), which recognize distinct phosphoepitopes of tau. Interestingly, the Alz50 antibody also shows a similar staining pattern (Fig. 5*C*).

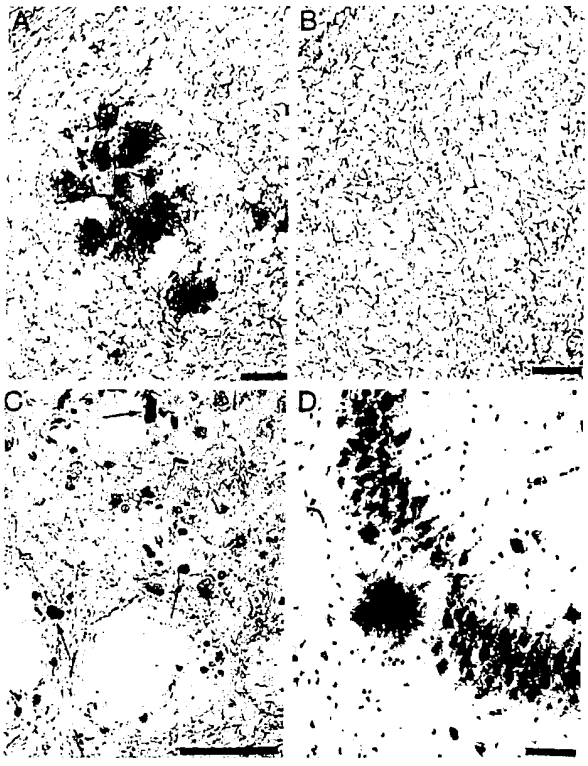

FIG. 4. Degenerative processes in APP 23 mice. (*A* and *B*) Staining for acetylcholinesterase in 12-month-old transgenic and control mice, respectively. A local distortion of cholinergic fibers in the plaque vicinity (*A*) compared with normal animals (*B*) can be noted. Neuritic spheroids (arrows) are stained with the neurofilament antibody NF200 (*C*). The loss of pyramidal neurons in the vicinity of Aβ deposits in area CA3 is shown in *D* by toluidine blue staining. (Bars = 50 μm.)

These antibodies do not stain sections from nontransgenic littermate controls. We failed to detect neurofibrillary tangles by the method of Gallyas.

Hyperphosphorylated Tau Can Be Detected Biochemically in Brain Extracts of APP 23 Mice. The increased phosphorylation of tau can also be demonstrated on immunoblots of brain extracts from APP 23 mice at 6 months and 15 months of age incubated with the AT8 (Fig. 6*A*) and the R27 antibody (data not shown). Age-matched littermate controls show much weaker immunoreactivity that does not increase with age. When incubated with phosphorylation-independent antibody tau7, no up-regulation of total tau protein is seen in transgenic mice (Fig. 6*B*). The tau phosphorylation therefore appears to occur parallel to Aβ peptide deposition.

DISCUSSION

Transgenic technology has led to recent advances in the generation of animal models developing aspects of AD pathology (20, 21). In this study we describe transgenic mouse lines overexpressing human APP with AD-linked mutations in a neuron-specific manner.

Increasing the total expression of human APP carrying the Swedish double mutation to 7-fold above endogenous APP leads to an early formation of amyloid plaques in neocortex and hippocampus as demonstrated in mice from line APP 23. A more than 10-fold overexpression was reported by Games *et al.* (20), whereas Hsiao and colleagues (21) describe transgene-derived APP levels exceeding endogenous APP by 6-fold. Interestingly, different promoter constructs, APP isoforms and FAD mutations were used in each study. Although it is

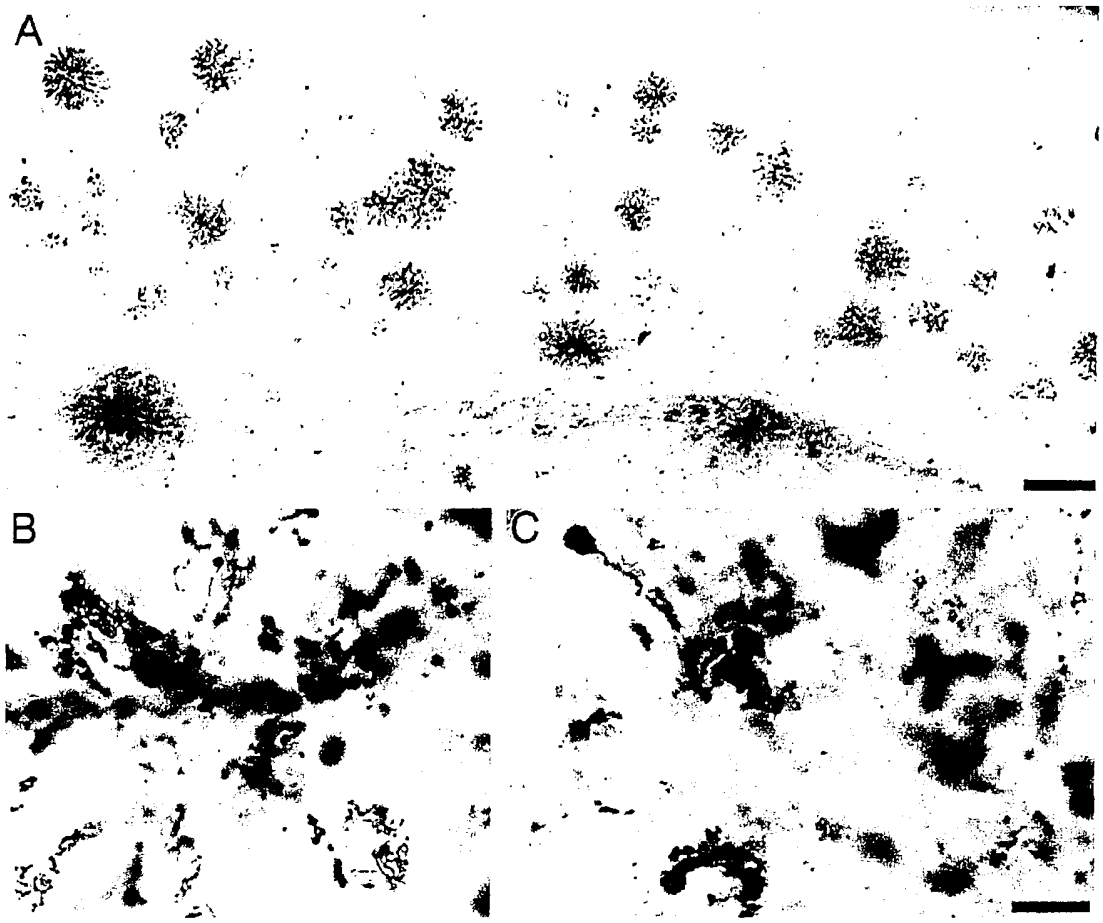

FIG. 5. Phosphorylation of tau in the brains of 12-month-old APP 23 transgenic mice. Immunocytochemistry with antibody AT8 was performed on free floating sections (40 μm) (*A* and *B*). Immunostaining reveals fiber-like structures resembling distorted neurites. Qualitatively similar patterns are obtained with the Alz50 antibody (*C*). [Bars = 100 μm (*A*) and 10 μm (*B* and *C*).]

difficult to compare the quantification of transgene expression in the individual studies, it is tempting to speculate that exceeding a certain threshold expression of human APP with a FAD-associated mutation is the trigger for plaque-like pathology.

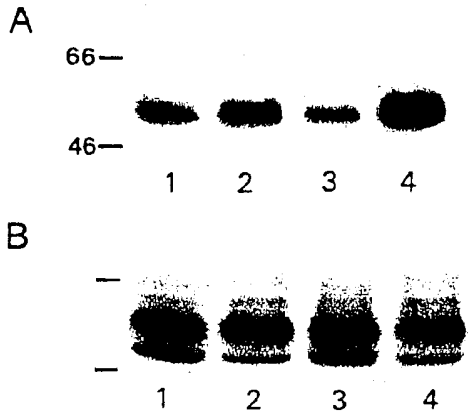

FIG. 6. Biochemical detection of hyperphosphorylated tau protein. Western blots of brain extracts from APP 23 mice, 6 months (lane 2) and 15 months (lane 4) of age and littermate controls (lanes 1 and 3) are shown in *A* and *B*. Blots were stained with antibodies AT8 (*A*) and tau7 (*B*). Numbers indicate molecular weights of marker proteins in kDa.

Our results also demonstrate that the combination of two genetic lesions in the transgenic mice leads to the development of AD plaques at a significantly lower expression level. Mouse line APP 22 overexpressing APP 2-fold matches the quantitative and spatial expression of line APP 14 (19). APP 14 mice carry the Swedish double mutation only and do not develop any obvious pathology up to 2 years of age. APP 22 mice, however, which express human APP with the combined Swedish (8) and London (9) mutations, develop plaques by 18 months of age. These data also suggest a major contribution of the Aβ-(1–42) species to the development of AD pathology at a given expression level.

Interestingly, the majority of Aβ deposits in APP 22 mice is of a diffuse type with a few congophilic plaques in hippocampus and neocortex. In contrast, APP 23 mice develop almost exclusively congophilic plaques already at their first appearance. These results may be difficult to reconcile with the proposed hypothesis (1) that deposits of the diffuse type form a precursor for congophilic neuritic plaques. The two described mouse lines, therefore, offer the opportunity to study such proposed plaque maturation processes.

When examined for additional characteristics, the pronounced glial reaction is apparent in both lines. As in AD, proliferating astrocytes can be detected throughout the brain and accumulate around deposits (36). Phosphotyrosine, MAC-1, MHC class II, and complement C3 immunoreactivity is also found. Although considered indicative of activated microglia in the brains of AD patients (37), the precise cellular

origin of these markers requires further investigation. These mice present a suitable model to investigate the pathways of inflammatory processes after Aβ formation in detail.

Besides the general detection of dystrophic neurites in the periphery of plaques by anti-neurofilament and anti-APP immunoreactivity, a local distortion of cholinergic fibers can be observed. The degeneration of cholinergic neurites and the association of cholinesterase activity with senile plaques is another well-described feature of AD (6, 7, 38) that is reproduced in the transgenic mice, suggesting a causal link between Aβ deposition and cholinergic degeneration.

Most importantly, hyperphosphorylated tau can be detected in distorted neurites associated only with congophilic plaques in both transgenic mouse lines with several antibodies recognizing distinct phosphoepitopes of tau. Fibrillary but not amorphous Aβ has been reported to induce hyperphosphorylation of tau and loss of microtubule binding in neuronal primary cultures (39). Similar changes in tau phosphorylation have also been described as early indicators of neurofibrillary changes in AD patients (40). The immunoreactivity with the Alz50 antibody is further evidence for tau-related pathology. This antibody was originally described to recognize an AD-specific epitope (35) and is now considered to react with a discontinuous epitope of tau that is formed after a conformational change of tau associated with polymerization into filaments (41). Therefore, one may expect neurofibrillary pathology in these mice despite the lack of tangles. This notion needs to be confirmed by ultrastructural analysis. A detailed ultrastructural study on APP transgenic mice with pathology (20), however, did not provide evidence for paired helical filaments (42).

Our two transgenic mouse lines develop distinct features of AD pathology and provide model systems to systematically investigate the role of the Aβ peptide in the pathogenesis of AD.

H. van der Putten supplied the murine Thy-1 expression cassette. M J. was supported by grants from the Swiss National Science Foundation.

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Related Articles, Links

Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production.

Citron M, Oltersdorf T, Haass C, McConlogue L, Hung AY, Seubert P, Vigo-Pelfrey C, Lieberburg I, Selkoe DJ.

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Progressive cerebral deposition of the 39-43-amino-acid amyloid beta-protein (A beta) is an invariant feature of Alzheimer's disease which precedes symptoms of dementia by years or decades. The only specific molecular defects that cause Alzheimer's disease which have been identified so far are missense mutations in the gene encoding the beta-amyloid precursor protein
(beta-APP) in certain families with an autosomal dominant form of the disease (familial Alzheimer's disease, or FAD). These mutations are located within or immediately flanking the A beta region of beta-APP, but the mechanism by which they cause the pathological phenotype of early and accelerated A beta deposition is unknown. Here we report that cultured cells which express a beta-APP complementary DNA bearing a double mutation (Lys to Asn at residue 595 plus Met to Leu at position 596) found in a Swedish FAD family produce approximately 6-8-fold more A beta than cells expressing normal beta-APP. The Met 596 to Leu mutation is principally responsible for the increase. These data establish a direct link between a FAD genotype and the clinicopathological phenotype. Further, they confirm the relevance of the continuous A beta production by cultured cells for elucidating the fundamental mechanism of Alzheimer's disease.

leaized rural life. 2. *Mus.* A pastorale. **—pas'tor·al·ism** **'tor·al·ist** *n.* **—pas'tor·al·ly** *adv.*

ile (pă'tə-răl', -răl', -ră'lē, pä'-) *n., pl.* **-ra·li** tal. < *pastorale,* pastoral < Lat. *pastoralis* < ... e PASTOR.] *Mus.* 1. An opera or other vocal composition ... rural subject or theme. 2. An instrumental composition ... ender melody in a moderately slow rhythm suggestive of ... al life.

ate (păs'tər-ĭt) *n.* 1. The office, rank, or jurisdiction of ... A pastor's term of office with one congregation. 3. A pastorate...

i·um pă-stôr'ē-əm, -stōr'-) *n.* *Chiefly Southern U.S.* nce of a pastor : PARSONAGE.

iciple *n.* A verb form indicating past or completed action ... it is used as a verbal adjective in phrases such as *completed* ... *molded clay* and with auxiliaries to form the passive voice and pluperfect tenses in constructions such as *The* ... *leted* and *The sculptor had molded the clay.*

ect *n.* The pluperfect.

ai (pə-strä'mē) *n.* [Yiddish < Rom. *pastramă* < ...] A highly seasoned smoked cut of beef, usu. from the breast ...

as'trē) *n., pl.* **-tries.** [< PASTE¹.] 1. A usu. rich, baked ... our, water, and shortening used for the crusts of foods such ... turnovers. 2. Baked foods made with pastry.

se *n.* A verb tense used to express an action or condition ... ed in or during the past, e.g., in *While I was working, the* ... *e news,* the verbs *was working* and *watched* are in the past ...

ge (păs'chər-ĭj) *n.* 1. The vegetation, as grass, eaten by ... mals. 2. a. Land covered with grass or vegetation suitable ... animals. b. The right to graze animals on such land. 3. The grazing cattle.

(păs'chər) *n.* [ME < OFr. < LLat. *pastura* < Lat. *pascere,* to ...] a. Vegetation, as grass, eaten as food by grazing animals. b. ... which such vegetation grows. 2. The feeding or grazing ... *v.* **-tured, -tur·ing, -tures.** *—vt.* 1. To herd animals ... re to graze. 2. To provide (animals) with pasturage. *—vi.* To graze in a pasture. **—pas'tur·a·ble** *adj.* **—past...**

land (păs'chər-lănd') *n.* Land suitable for grazing.

'stē) *adj.* **-i·er, -i·est.** 1. Like paste in color or consistency ... aving a pale and lifeless appearance. **—past'i·ness** *n.*

ăs'tē) *n., pl.* **-ties.** [ME < OFr. *pastee* < *paste,* paste.] A ... filling of seasoned meat or fish.

d history: The noun *pa.ty*, "a meat pie," was borrowed ... ench *pastee* in the 13th century. *Pastee* is derived from Old ... *te,* literally "paste," which originally referred to an edible ... our and liquid. Old French *pastee* is descended from the ... as Italian *pasta.* The modern French form of *pastee* is *pâté* ... borrowed into English in the 18th century as a synonym for ... in English now denotes the meat alone, without its pastry ... is an anglicized variant of *pâté.*

n (pē'ā') *n.* A public-address system.

pat·ted, pat·ting, pats. [ME. *patte,* a light blow.] To tap gently with the open hand or with something flat ... e lightly as a gesture of affection. 2. To mold by tapping ... the hands or a flat implement <*pat* dough into a pan> ... run or walk with a tapping sound. 2. To hit or beat against ... gently or lightly. *—n.* 1. A light stroke or tap. 2. The sound ... ight stroke or tap or by light footsteps. 3. A small mass of ... as butter, shaped into an individual portion. *—adj.* 1. Op... itting. 2. Needing no change : exactly right. 3. Contrived ... with a *pat* reply> *—adv.* *Informal.* 1. Without changing : READFASTLY <stood *pat* on my decision> 2. Perfectly ... ty. **—down pat.** *Informal.* Known and understood com... **pat'ly** *adv.* **—pat'ness** *n.*

pə-tä'kə *n.* [Port.] —See table at CURRENCY.

um (pə-tā'jē-əm) *n., pl.* **-gi·a** (-jē-ə) [NLat. < Lat., gold ... woman's tunic < Gk. **patageion* < *patagos,* clatter.] 1. ... n membrane extending between the fore and hind limbs to ... g or winglike extension, as in flying squirrels and bats. 2. ... ble membranous fold of skin between the wing and body of ...

h) *n.* [ME *pacche.*] 1. A small piece of material affixed to ... ger piece to reinforce or conceal a weakened or worn area. ... l piece of cloth used for patchwork. b. A small cloth badge ... sleeve to indicate the military unit to which one belongs. ... ng or dressing applied to protect a wound or sore. 4. A small ... d of cloth worn over an injured eye. 5. A beauty spot. 6. a. ... ce of land. b. The produce grown on such a piece of land <a ... umbers> 7. A small part or section of a surface that differs ... trasts with the whole <The snow made white patches on the ... nt.> 8. A small piece or portion. *—vt.* **patched, ...**

...patch·es. 1. To put a patch or patches on. 2. a. To make (e.g., ...) by sewing scraps of material together. b. To make by piecing ... elements together, esp. hastily <*patched* together a report> 3. To mend, repair, or put together, esp. hastily or shoddily. ... To settle <We *patched up* our differences.> **—patch'a·ble** *adj.* **—patch'er** *n.*

patch·ou·li *also* **patch·ou·ly** *or* **pach·ou·li** (păch'ə-lē, pə-choo'lē) *n., pl.* **-lis** *also* **-lies** *or* **-lis.** [Tamil *paccilai.*] 1. An Asiatic ... of the genus *Pogostemon,* esp. *P. patchouly* or *P. cablin,* bearing leaves that yield a fragrant oil used in perfumery. 2. A perfume made from the oil of the patchouli.

patch pocket *n.* An unfitted flat outside pocket on a garment.

patch test *n.* A test for allergic reaction made by applying a suspected allergen to the skin in a small surgical pad.

patch·work (păch'wûrk') *n.* 1. Needlework made of varicolored patches of material sewed together, as in a quilt. 2. A miscellany : jumble.

patch·y (păch'ē) *adj.* **-i·er, -i·est.** 1. Made up of or marked by patches <*patchy* jeans> 2. Uneven in quality or performance. **—patch'i·ly** *adv.* **—patch'i·ness** *n.*

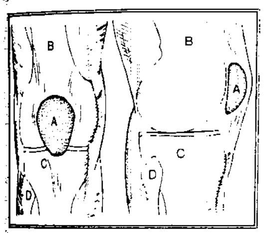
pate (pāt) *n.* [ME.] 1. The head, esp. the top of the head <a shiny pate> 2. The brain : intellect. **—pat'ed** *adj.*

pâte (pät) *n.* [Fr. < OFr. *paste,* paste.] Paste used in making pottery and porcelain.

pâ·té (pä-tā') *n.* [Fr. < OFr. *pastee,* pasty (pie), < *paste,* paste.] 1. A meat paste, esp. pâté de foie gras. 2. A small pastry filled with meat or fish.

pâ·té de foie gras (pä-tā' də fwä grä') *n.* [Fr. : *pâté,* pâté + *de,* of + *foie,* liver + *gras,* fat.] A paste made from goose liver, usu. with truffles.

pa·tel·la (pə-tĕl'ə) *n., pl.* **-lae** (-ē) [Lat. < dim. of *patina,* plate. —see PATEN.] 1. a. A flat triangular bone at the front of the knee joint. b. A dish-shaped anatomical formation. 2. An ancient Roman pan or dish. **—pa·tel'lar, pa·tel'late** (-ĭt, -āt') *adj.*



patella
A. patella, B. femur, C. tibia, D. fibula

pa·tel·li·form (pə-tĕl'ə-fôrm') *adj.* PATELL(A) + -FORM.] Shaped like a pan, dish, or cup.

pat·en *also* **pat·in** (păt'n) *or* **pa·tine** (pă-tēn') *n.* [ME < OFr. *patene* < Lat. *patina,* plate < Gk. *patanē.*] 1. A plate used to hold the Eucharistic bread. 2. A plate. 3. A thin metal disk.

pa·ten·cy (păt'n-sē) *n.* The quality or state of being obvious.

pat·ent (păt'nt) *n.* [ME unsealed < OFr. < Lat. *patens* < pr.part. of *patere,* to be opened.] 1. a. A grant made by a government to an inventor, assuring the sole right to make, use, and sell the invention for a certain period of time. b. Letters patent. c. Something protected by such a grant. 2. a. A grant made by a government to an individual, conveying fee-simple title to public lands. b. The official document of such a grant. c. The land so granted. 3. An exclusive title or right. *—adj.* 1. Plain : obvious. 2. Protected by a patent. 3. Of, relating to, or dealing in patents. 4. (păt'nt) *Biol.* Spreading open : EXPANDED. 5. Of high quality. —Used of flour. *—vt.* **-ent·ed, -ent·ing, -ents.** 1. To obtain a patent on. 2. To grant a patent to. **—pat'ent·a·bil'i·ty** *n.* **—pat'ent·a·ble** *adj.* **—pat'ent·ly** (păt'nt-lē) *adv.*

pat·ent·ee (păt'n-tē') *n.* One who has been granted a patent.

patent leather (păt'nt) *n.* [So called because it is made by a once-patented process.] 1. Black leather finished to a hard, shiny surface. 2. Any of several synthetic materials resembling patent leather.

patent log *n.* *Naut.* A screw log.

patent medicine *n.* A medical preparation, as a drug, that is protected by a patent and can be purchased without a prescription.

Patent Office *n.* A government bureau that studies claims for and grants patents.

pat·en·tor (păt'n-tər, păt'n-tôr') *n.* One that grants a patent.

patent right *n.* The right granted by a patent, esp. the right to have exclusive manufacture and sale of an invention.

pa·ter (pā'tər) *n.* [Lat.] *Chiefly Brit.* Father.

pa·ter·fa·mil·i·as (pā'tər-fə-mĭl'ē-əs, pä'tər-) *n., pl.* **pa·tres·fa·mil·i·as** (pā'trēz-fə-mĭl'ē-əs, pä'-) [Lat. : *pater,* father + *familia,* family.] 1. The male head of a household. 2. The father of a family.

pa·ter·nal (pə-tûr'nəl) *adj.* [Med. Lat. *paternalis* < Lat. *paternus* < *pater,* father.] 1. Of, relating to, or typical of a father : FATHERLY. 2. Inherited or received from a father. 3. Of or relating to the father's side of a family. **—pa·ter'nal·ly** *adv.*

pa·ter·nal·ism (pə-tûr'nə-lĭz'əm) *n.* A policy or practice of treating or governing people in a paternal manner, esp. by taking care of their needs without giving them responsibility. **—pa·ter'nal·is'tic** *adj.* **—pa·ter'nal·is'ti·cal·ly** *adv.*

pa·ter·ni·ty (pə-tûr'nĭ-tē) *n.* [OFr. *paternite* < LLat. *paternitas* < Lat. *paternus,* paternal < *pater,* father.] 1. The fact or condition of being a father. 2. Paternal descent. 3. Origin : authorship.

paternity test *n.* A test using blood group identification of a mother, child, and suspected father to establish probable paternity.

pa·ter·nos·ter (pā'tər-nŏs'tər, pä'tər-, păt'ər-) *n.* [ME < Med. Lat. < Lat. *pater noster,* our father.] 1. often **Paternoster.** The Lord's Prayer. 2. One of the large beads on a rosary on which the Lord's Prayer is said. 3. A sequence of words spoken as a prayer or magic formula. 4. A weighted fishing line with several jointed attachments for hooks connected by beadlike swivels.

path (păth, päth) *n., pl.* **paths** (păthz, päthz, păths, päths) [ME < OE *pæth.*] 1. A track or way made by footsteps. 2. A road or way made for a particular purpose <a hiking *path*> 3. The route or course along which something moves <the *path* of a cyclone> 4. A course of action or conduct <the *path* of virtuousness> 5. *Computer Sci.* A list of the complete hierarchical structure of a file.

path– *pref.* var. of PATHO-.

–path *suff.* [back-formation < -PATHY.] 1. A practitioner of a given type of medical treatment <naturo*path*> 2. One suffering from a given type kind of disorder <socio*path*>

Pa·than (pə-tän') *n.* [Hindi *Paṭhān* < Pashto *Pĕṣtana,* pl. of *Pĕṣtūn,* an Afghan < *pashtu,* Pashto.] A member of a Pashto-speaking people of eastern Afghanistan and northwestern Pakistan, constituting the majority population of Afghanistan.

pa·thet·ic (pə-thĕt'ĭk) *also* **pa·thet·i·cal** (-ĭ-kəl) *adj.* [Fr. *pathétique* < LLat. *patheticus* < Gk. *pathētikos,* sensitive < *pathētos,* liable to suffer < *pathos,* suffering.] 1. Of, relating to, expressing, or arousing pity, sympathy, or tenderness. 2. Inadequate : pitiful. <a *pathetic* attempt at humor> **—pa·thet'i·cal·ly** *adv.*

pathetic fallacy *n.* The attribution of human emotions or characteristics to things, e.g. *angry sea, kind rain.*

path·find·er (păth'fīn'dər, päth'-) *n.* One who discovers a way into or through unexplored regions.

path·less (păth'lĭs) *adj.* Unmarked by paths or trails.

patho– *or* **path–** *pref.* [NLat. < Gk. < *pathos,* suffering.] Disease : suffering <*pathogen*>

path·o·gen (păth'ə-jən) *n.* An agent that causes disease, esp. a microorganism such as a bacterium or fungus.

path·o·gen·e·sis (păth'ə-jĕn'ĭ-sĭs) *also* **pa·thog·e·ny** (pə-thŏj'ə-nē) *n.* The development of a diseased or morbid condition.

path·o·gen·ic (păth'ə-jĕn'ĭk) *also* **path·o·ge·net·ic** (păth'ō-jə-nĕt'ĭk) *adj.* Capable of causing disease. **—path'o·gen'i·cal·ly** *adv.* **—path'o·ge·nic'i·ty** (-jə-nĭs'ĭ-tē) *n.*

pa·thog·no·mon·ic (pə-thŏg'nə-mŏn'ĭk, păth'ăg-nō-) *adj.* [Gk. *pathognōmonikos* : *pathos,* suffering + *gnōmonikos,* able to judge < *gnōmōn,* interpreter.] Typical of a particular disease.

path·o·log·i·cal (păth'ə-lŏj'ĭ-kəl) *also* **path·o·log·ic** (-ĭk) *adj.* 1. Of or relating to pathology. 2. Relating to or caused by disease. 3. Disordered in behavior. **—path'o·log'i·cal·ly** *adv.*

pa·thol·o·gy (pă-thŏl'ə-jē) *n., pl.* **-gies.** 1. The branch of medicine concerned with the study of the nature of disease and its causes, processes, development, and consequences. 2. Anatomic or functional manifestations of disease. **—pa·thol'o·gist** *n.*

pa·thos (pā'thŏs', -thôs') *n.* [Gk., suffering.] 1. A quality arousing feelings of pity, sympathy, tenderness, or sorrow. 2. A feeling of sympathy or pity. 3. The transient, emotional, or subjective elements in a work of art.

path·way (păth'wā', päth'-) *n.* A path.

–pathy *suff.* [Lat. *-pathia* < Gk. *-patheia* < *pathos,* suffering.] 1. Feeling : suffering : perception <tele*pathy*> 2. a. Disease <neuro*pathy*> b. A system of treating disease <homeo*pathy*>

pa·tience (pā'shəns) *n.* 1. The quality of being patient : capacity of calm endurance. 2. *Chiefly Brit.* SOLITAIRE 2.

pa·tient (pā'shənt) *adj.* [ME *pacient* < OFr. < Lat. *patiens* < *pati,* to endure.] 1. Capable of bearing affliction calmly. 2. Understanding : tolerant. 3. Persevering : constant <a *patient* student> 4. Capable of bearing delay. *—n.* 1. One under medical treatment. 2. *Archaic.* One who suffers. **—pa'tient·ly** *adv.*

★ ***syns:*** PATIENT, FORBEARING, LONG-SUFFERING, RESIGNED *adj.* *core meaning* : enduring or capable of enduring hardship or inconvenience without complaint <a *patient* parent> ***ant:*** IMPATIENT

pat·in (păt'n) *n.* var. of PATEN.

pat·i·na¹ (păt'ə-nə) *n., pl.* **-nae** (-nē) [Med. Lat. < Lat., plate. —see PATEN.] PATEN 3.

pat·i·na² (păt'ə-nə, pə-tē'nə) *also* **pa·tine** (pă-tēn') *n.* [Ital. < Lat., plate. —see PATEN.] 1. A thin layer of usu. brown or green corrosion that appears on copper or copper alloys, as bronze, as a result of natural or artificial oxidation. 2. The sheen on an antique surface produced by use and age.

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Amyloid deposition as the central event in the aetiology of Alzheimer's disease

John Hardy and David Allsop

While there may be many causes of Alzheimer's disease (AD), the same pathological sequence of events, described here by John Hardy and David Allsop, *is likely to occur in all cases. The recent discovery of a pathogenic mutation in the β-amyloid precursor protein (APP) gene on chromosome 21 suggests that APP mismetabolism and β-amyloid deposition are the primary events in the disease process. The occurrence of AD in Down syndrome is consistent with this hypothesis. The pathological cascade for the disease process is most likely to be: β-amyloid deposition → tau phosphorylation and tangle formation → neuronal death. The development of a biochemical understanding of this pathological cascade will facilitate rational design of drugs to intervene in this process.*

Alzheimer's disease (AD) is the fourth major cause of death in the developed world after heart disease, cancer and stroke. It is largely, but not exclusively, a disease of the elderly and has been estimated to afflict 1–6% of those aged over 65 years. Demographic changes (the falling birth rate and ageing of the baby boom that followed World War 1) mean that the prevalence of the disease is increasing. Typically, AD begins insidiously with memory problems, which become progressively worse until sufferers are bedridden, doubly incontinent, and have completely lost their presymptomatic persona.

Clearly this disease is a major social and health care problem. There is no effective treatment, and for the majority of patients there is no certain means of diagnosis other than brain biopsy. It is only recently that advances in molecular genetics have begun to shed some light on the causes of the disease, although it has long been recognized that persons with trisomy 21 (Down syndrome) inevitably develop the full neuropathological changes of advanced AD by their fourth or fifth decade.

The finding of an amyloid precursor protein (APP) gene mutation in familial AD (see below) now makes it clear that deficits in neurotransmitters, transmitter-metabolizing enzymes and receptors are a consequence of amyloid deposition (or abnormal APP processing) and not the initial cause of the disease. Therapeutic approaches to AD based on increasing the levels of acetylcholine in the brain have not met with great success, despite claims to the contrary, and drug development in the future may turn towards compounds designed to inhibit the formation of amyloid toxic activity of abnormal APP fragments, or interfere with the sequence of events linking amyloid deposition and aberrant APP processing to neuritic alteration, neurofibrillary tangle formation and cell death.

If this type of therapeutic approach could be applied in the early stages of the disease, before significant damage has accrued to neuronal systems, then there is good reason to hope that this might halt or slow the progression of the disease. For these reasons, an understanding of the role of amyloid formation and senile plaque development in AD is of considerable importance to pharmacologists.

Pathological features

The pathology of the disease is complex. There are three well-known sites of abnormal fibrous protein deposits within the brains of AD patients. These are the senile plaques, the neurofibrillary tangles and the walls of cerebral blood vessels. There is also extensive neuronal damage and loss. All of these features are also found, usually to a lesser degree, in the 'normal' elderly. Thus the relationship between AD and 'normal' ageing remains unclear. There has been much debate about the relative importance of these different pathological features and how they relate to each other. This article reviews the present understanding of the molecular pathology of these lesions and the aetiology of the disease.

Senile plaques

The classical senile plaque consists of a central core of radiating amyloid fibrils surrounded by a rim of dystrophic neurites together with reactive microglia and

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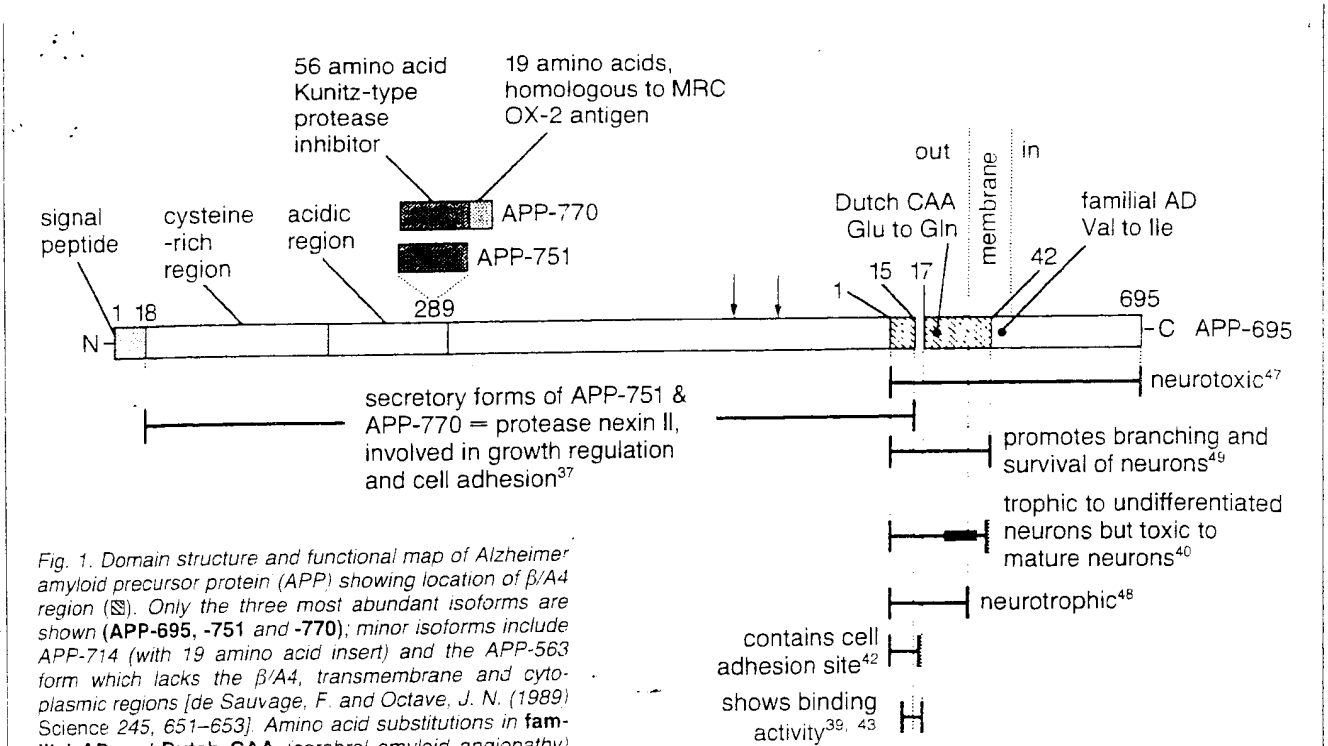

Fig. 1. Domain structure and functional map of Alzheimer amyloid precursor protein (APP) showing location of β/A4 region (▨). Only the three most abundant isoforms are shown **(APP-695, -751** *and* **-770)**; *minor isoforms include APP-714 (with 19 amino acid insert) and the APP-563 form which lacks the β/A4, transmembrane and cytoplasmic regions [de Sauvage, F. and Octave, J. N. (1989) Science 245, 651–653]. Amino acid substitutions in* **familial AD** *and* **Dutch CAA** *(cerebral amyloid angiopathy) are indicated, and two* **arrows** *mark glycosylation sites.*

term used to describe a group of chemically heterogeneous proteins found in a number of different diseases and tissues. All amyloid deposits are composed at the ultrastructural level of straight, unbranching fibrils of 6–10 nm diameter, and the deposits also share common properties of Congo red birefringence and resistance to proteolysis. These properties have been ascribed to a predominant cross-β structure in the constituent polypeptide chains.

Amino acid sequencing of senile plaque amyloid A4 protein[1] revealed it to be essentially the same as the cerebrovascular amyloid β protein originally isolated from meningeal blood vessels by Glenner and Wong[2]. The 39–42 amino acid senile plaque or cerebrovascular amyloid peptide will be referred to here as β/A4.

Molecular cloning has indicated that β/A4 is a small fragment of a much larger amyloid precursor protein (APP)[3] (see Fig. 1). This precursor is predicted to have a single membrane-spanning region with a long extracellular N-terminal segment and a short intracellular C-terminal tail. The β/A4 sequence begins close to the membrane on the extracellular side and ends part-way through the putative transmembrane region.

There are at least five alternative transcripts of the APP gene, some of which contain an internal Kunitz-type protease inhibitor insert[4]. Significantly, the APP gene was localized by several groups to chromosome 21. Possible functions of APP and its role in the pathogenesis and aetiology of AD are considered further below.

Over the past few years considerable attention has been focused on a previously poorly described type of plaque-like lesion that cannot be detected easily using routine Congo red, thioflavin or silver stains. These 'diffuse' plaques can be seen as an area of granular staining after an immunohistochemical reaction with antibodies to β/A4. Most diffuse plaques show no association with abnormal neurites or reactive glial cells. They are often the only form of senile plaque found in cases of Down syndrome below the age of 35 (Ref. 5), and substantial numbers have even been detected in a 13 year old Down syndrome patient[6]. Ultrastructural observations of these lesions have revealed few or no amyloid fibrils, leading to the proposal that they may be accumulations of 'pre-amyloid' (β/A4, APP or fragments of APP not yet in a fibrous form)[7].

These observations suggest that diffuse plaques are an early stage of plaque formation and that the deposition of β/A4 (or its precursor) precedes any obvious neuritic pathology. Abundant diffuse plaques have also been found in the brains of ex-boxers with dementia pugilistica[8]. In AD, diffuse plaques are usually more numerous and widespread throughout the CNS than typical senile plaques (e.g. they are often found in the spinal cord and cerebellum) implying that they can evolve into typical neuritic plaques only in specific brain regions[9,10].

The dystrophic neurites surrounding the amyloid core of a mature senile plaque contain paired helical filaments (PHFs) – pairs of filaments 10 nm in diameter, wound into a left-handed helix with a cross-over approximately every 80 nm. These PHFs also constitute the main structural element of the neurofibrillary tangle[11].

Neurofibrillary tangles and neuropil threads

Neurofibrillary tangles of PHFs are found inside dying neurons. There has been much debate and

confusion concerning the biochemical make-up of these lesions. Direct protein chemical analyses of isolated PHF preparations have identified ubiquitin, β/A4 and the microtubule-associated protein, tau, as potential constituents, while immunohistochemical studies have shown that neurofibrillary tangles share epitopes with these proteins and with neurofilament components and another microtubule-associated protein, MAP 2 (reviewed in Ref. 12).

It has been suggested that PHFs are composed of β/A4, but this remains highly controversial. It is now clear that some extracellular 'tombstone' tangles (where the surrounding neuron has completely degenerated) do indeed react with antibodies to β/A4 (Ref. 13). However, recent ultrastructural observations suggest that this finding is due to the secondary deposition of amyloid fibrils on the surface of exposed tangles rather than any intrinsic contribution of β/A4 to PHF structure[14].

A report appearing to confirm that intracellular tangles can be immunostained with antibodies to β/A4[15] must also be treated with caution, since this finding could be due to APP within the cell, possibly trapped inside or adsorbed onto the surface of the tangle. APP is synthesized in the neuronal cell body and normally undergoes fast anterograde axonal transport[16]. Large bundles of PHF in the cytoplasm might interfere with this process and lead to an abnormal accumulation of APP within the perikarya of affected cells. This might also explain the finding of Yamaguchi *et al.*[17], who have recently described APP-like immunoreactivity of intracellular tangles.

Many of the chemical studies on tangle composition suffer from the lack of sufficient quantitative data on protein recovery and yields of amino acid residues obtained during sequencing. Thus it is not always clear if the reported amino acid sequences relate to major PHF components or minor contaminants (e.g. co-purifying amyloid fibrils). The most convincing data suggest that PHFs consist, at least in part, of an abnormally phosphorylated fragment of one or more isoforms of tau protein[18]. However, it has been claimed that about 10% of the mass of PHFs, and the remainder is unknown[12]. There is scope for further protein chemical analyses of PHFs.

A third site of PHF accumulation in the brain in AD is inside tau-immunoreactive, slender, argyrophilic fibres found scattered throughout the allocortical and isocortical neuropil. Some of these 'neuropil threads'[19] or 'curly fibres'[20] have been shown to be the dendrites of tangle-bearing neurons[21]; however, they are considered by Ihara[20] to be dendritic growth cones attempting a massive regenerative response.

Neuronal loss

The distribution of neuronal damage and loss has never been thoroughly and formally quantified; the clearest description of this pathology is given by Brun and colleagues (reviewed in Ref. 22). Two important points may be gleaned from the distribution of neuronal loss: the first is that the affected neurons do not share any particular transmitter or any other biochemical marker tested so far (deficits have been found in cholinergic, noradrenergic, serotonergic and dopaminergic systems, as well as in amino acid and peptide neurotransmitters); the second is that the selectivity of neuronal loss appears to be anatomically determined. In particular, the suggestion that the pathology spreads along neuronal pathways is a particularly attractive way to explain the distribution of neuronal damage and loss[22].

Aetiology of Alzheimer's disease

A large number of epidemiological studies have consistently shown that family history is a risk factor for developing AD[23]. In addition there are numerous reports of families in which Alzheimer's disease is inherited as an autosomal dominant disorder (reviewed in Ref. 24). Genetic linkage studies showed that the pathogenic locus for some families was on the proximal long arm of chromosome 21 (Ref. 25), although in other families there was no evidence for a lesion on this chromosome[26].

It is now clear that one locus on chromosome 21 is the APP gene, and that a mutation in this gene leucine for valine at codon 717 (residue 642 in APP-695) (Fig. 1) gives rise to the disease in some families[27]. An alternative mutation resulting in a substitution of glutamine for glutamic acid at codon 693 (residue 618 in APP-695) results in the deposition of β/A4 amyloid in the walls of cerebral blood vessels, invariably leading to death at an early age due to massive cerebral haemorrhage[28]. Patients with the latter disorder (cerebral amyloid angiopathy of Dutch type) sometimes also develop diffuse plaques in the brain but there are no significant numbers of typical senile plaques or neurofibrillary tangles. Neither of these APP mutations has been found in the general population, and the APP717 mutation has now been detected in English, North American and Japanese families with early onset AD[29].

It is likely that further alternative APP gene mutations remain to be discovered in other kindreds with autosomal dominant familial AD. Linkage with other genetic markers on or outside chromosome 21 could be explained by mutations in regions of DNA controlling APP gene expression or in genes coding for proteins having some influence on APP metabolism (e.g. APP-processing enzymes or their inhibitors).

It should be stressed that the proportion of cases in which AD is acquired as a single-gene, autosomal dominant disorder is very low and there is a substantial body of evidence indicating that the neuropathological changes of AD can also be triggered (most likely in genetically susceptible individuals) by various environmental factors. Potentially important factors include aluminium exposure, head trauma (c.f. boxers' brains[8] noted above), and virus infections. However, the fact that an APP gene mutation can give rise to all of the neuropathological hallmarks of AD without any preceding underlying neuronal pathology or any other defect strongly suggests that amyloid mismetabolism and deposition is the seminal event in the pathogenesis of all cases of AD.

Functions of APP and mechanism of β/A4 formation

APP is expressed at high levels

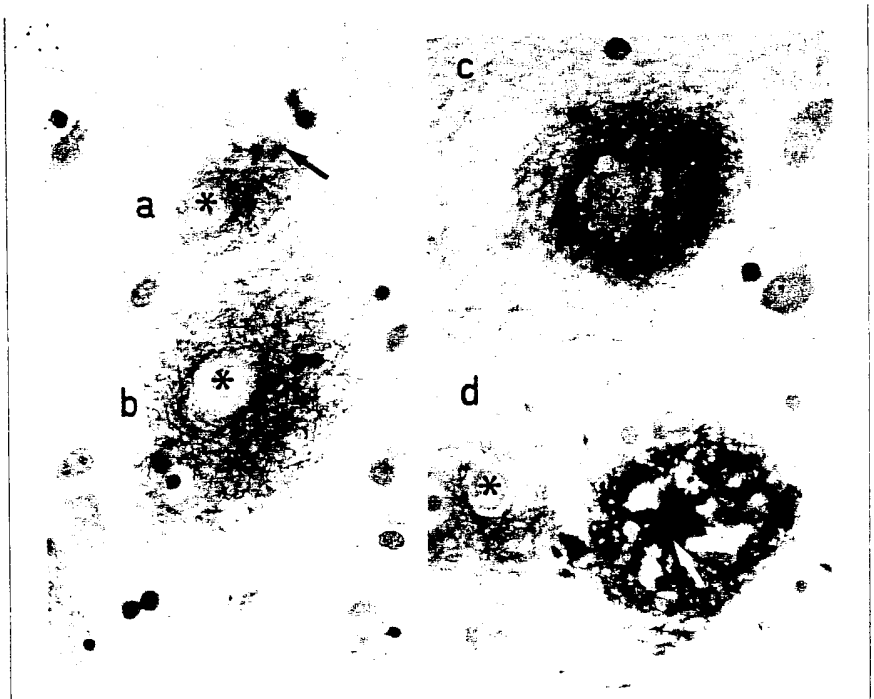

Fig. 2. Plaques from cases of Down syndrome, stained by a sensitive methenamine silver technique with Nissl counterstain. Note that amyloid or 'pre-amyloid' deposits in diffuse plaques **(a, b, c)** *are frequently seen to accumulate around neuronal cell bodies* **(asterisks)** *and dendrites, but show no obvious relationship with capillary blood vessels* **(black arrow)**. *These photographs show a possible progression of stages, the* **white arrow** *in* **d** *indicating the dense amyloid core of a fully-developed plaque.* Taken from Allsop, D. *et al.* (1989) *Neuropatnol. Appl. Neurobiol.* 15, 531–542, with permission.

out the body. In most tissues, transcript APP-770 is the most abundant, but in the CNS the transcript lacking the protease inhibitor domain (APP-695) is predominant. This leaves a number of important unanswered questions. What are the physiological functions of the various APP isoforms, and why is APP-695 so abundant in brain tissue? From which tissue source(s) and isoform(s) of APP is the amyloid in the brain derived? Where does the conversion of APP into amyloid take place and what are the molecular mechanisms involved? How do the APP gene mutations mentioned above lead to brain amyloidosis? These questions are presently the subject of intense investigation, and answers to some of them are only just beginning to emerge.

Secreted forms of APP

Soluble, secretory derivatives of APP can be produced by proteolytic cleavage within the β/A4 sequence close to the extracellular side of the membrane. These have been detected in cerebrospinal fluid[30,31], the conditioned media of various cell cultures[30], and in human serum[32]. Most of the secretory APP circulating in the blood probably originates from platelets[33,34]. Human embryonic kidney cells transfected with cDNA constructs encoding full-length forms of APP-695 and APP-751 release secretory forms of APP that terminate at residue Gln15 of β/A4, leaving behind a C-terminal membrane-bound fragment commencing at Leu17 (Ref. 35; Fig. 1). This suggests that an 'APP secretase' cleavage enzyme acts at either the Gln15–Lys16 or Lys16–Leu17 bonds, leaving Lys16 to be excised by an aminopeptidase or carboxypeptidase, respectively.

In AD an alternative or abnormal pathway of APP proteolysis (involving cleavage at two sites on either side of β/A4) must liberate the amyloid peptide. Experiments with synthetic β/A4 peptides indicate that the liberated peptide could spontaneously aggregate into amyloid fibrils (reviewed in Ref. 12). At present the identity of the proteolytic enzymes involved in the 'normal' and 'abnormal' APP-processing pathways is unknown, although some preliminary data on this subject have been reported (reviewed in Ref. 36).

Physiological functions of APP

These appear to include protease inhibition and a role in cell adhesion and the regulation of cell growth (Fig. 1). Secretory forms of APP containing the Kunitz inhibitor insert have the same N-terminal sequence, molecular mass and properties as protease nexin II, a growth-regulating molecule secreted by fibroblasts[37]. Secretory APP/nexin II can form stable complexes with several serine proteases, such as trypsin and chymotrypsin, and in the blood this species conceivably plays a role in the physiological events associated with blood clotting, by inhibiting appropriate coagulation pathway enzymes including coagulation factor XIa (Ref. 38).

Allsop *et al.*[39] suggested that the structural organization of APP resembles the epidermal growth factor precursor and speculated that the β/A4 peptide might show biological activity. Subsequently, synthetic β/A4 peptides of various lengths were shown to exhibit neurotrophic or neurotoxic activity (Fig. 1).

A recent report suggests that this property resides in residues 25–35 of β/A4, a region showing homology with the tachykinin family of neuropeptides[40]. A β/A4 25–35 peptide proved to be trophic at low concentrations to undifferentiated rat hippocampal neurons but toxic at higher concentrations to mature neurons.

These effects were mimicked by substance P antagonists and reversed by substance P agonists, implying that they are mediated by a tachykinin receptor[40]. Subsequently, the neurotoxic potency of β/A4 was reported to be greatly enhanced by nerve growth factor[41]. These reports await confirmation.

Intact APP promotes cell–substratum adhesion of both neuronal and non-neuronal cells. Such APP-mediated adhesion can be specifically inhibited by antibodies directed against residues 1–16 of β/A4, suggesting the presence of a cell adhesion site within this region[42]. This might also explain the earlier findings of binding sites for a β/A4 8–17 pep-

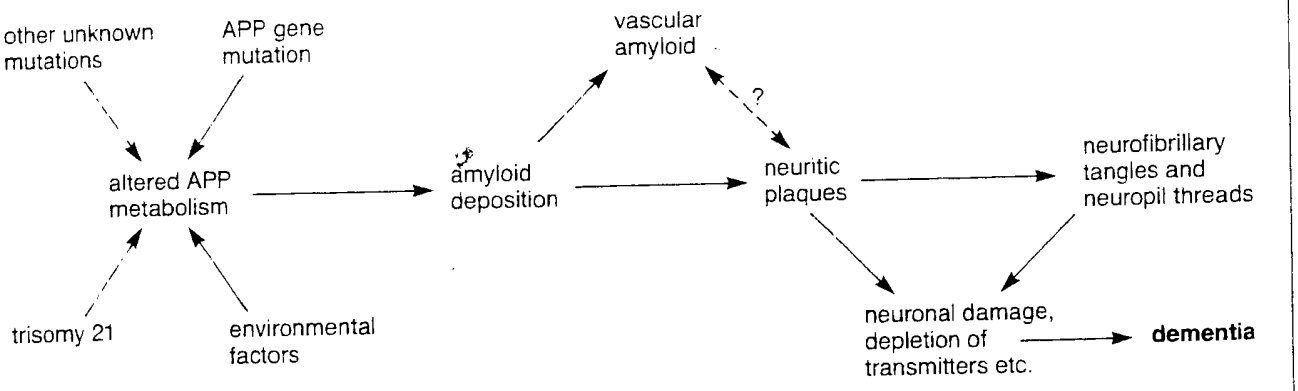

Fig. 3. Suggested pathogenic pathway for the progression of Alzheimer's disease.

tide in human adrenal gland and in the islets of Langerhans of the pancreas[34]. These observations have recently been extended to include high-affinity binding sites (with a K_d in the picomolar range) in rat brain[43]. Thus APP seems to be a molecule with at least three functionally important domains: protease inhibitor, growth regulatory site and cell adhesion site. The size and nature *in vivo* of all of the physiologically active fragments of APP that express these properties is not clear.

Amyloid deposition

In Down syndrome, amyloid deposition might be a consequence of an excessive amount of APP (due to a 50% increase in gene dosage) causing overloading of the normal proteolytic processing pathway and metabolic diversion into the alternative amyloid-generating pathway[6].

The APP693 mutation in cerebral amyloid angiopathy of Dutch type lies within six residues of the normal proteolytic cleavage site, while the APP717 mutation in familial AD is four residues past the C-terminus of the β/A4 region (Fig. 1). One simple hypothesis is that these mutations somehow directly influence APP proteolysis and lead to inappropriate degradation and β/A4 deposition.

An alternative explanation for the effects of the APP693 mutation is that the 'abnormal' processing pathway does in fact exist to some extent in normal brain, but the β/A4 peptide with the glutamate-to-glutamine substitution has a amyloid fibrils (i.e. is more 'amyloidogenic') than the normal peptide.

This second explanation appears not to apply to the APP717 mutation since it lies just outside the β/A4 region. However, the amyloid from familial cases of AD with this mutation has not yet been isolated and sequenced, and it is possible that the β/A4 peptide in these patients is extended beyond 42 residues to include the mutated amino acid. In this regard, it may be significant that in other chemical classes of amyloid where there is a point mutation (e.g. transthyretin, cystatin C) the deposited fibrils always contain the mutated residue. A major problem in determining the molecular effects of the APP amino acid substitutions will be that any alterations in processing need only be marginal to account for the slow build-up of β/A4 amyloid over decades.

The origin of the β/A4 amyloid is a contentious issue, with most advocates supporting either a vascular or a neuronal source for the APP. In the case of the senile plaque amyloid, the balance seems to be shifting decidedly in favour of a neuronal origin. APP is expressed at high levels in neurons, and the neuritic elements of typical senile plaques have been shown to react with antibodies to various regions of APP[44,45].

Observations of diffuse (presumably early) plaques have consistently failed to reveal any close spatial relationship with blood vessels. On the contrary, most diffuse plaques in Down syndrome and AD seem to be composed reactive material decorating neuronal soma and dendrites of local pyramidal neurons (Fig. 2).

Progression of disease

The fact that the typical senile plaque is intimately associated with abnormal neuritic elements containing PHF suggests that plaques and tangles do not arise independently but that formation of one leads to formation of the other. The discovery of an APP gene mutation in cases of familial AD with both senile plaques and neurofibrillary tangles implicates the former as the primary lesion.

This conclusion is supported by the temporal sequence of events deduced from cases of Down syndrome of different ages. Diffuse plaques are the first detectable lesion in Down syndrome[5,6] where they clearly pre-date neurofibrillary tangles, cerebrovascular amyloidosis, or any discernible neuritic change. Later, typical senile plaques with a surrounding neuritic response appear, and these are accompanied by neurofibrillary tangles and the closely associated neuropil threads. It is only later, after the age of 50, that any gross atrophy and loss of neurons are evident[46].

This allows a probable pathogenic pathway for AD to be delineated (Fig. 3) running from β/A4 amyloid formation and deposition, through neurofibrillary tangle formation, to cell loss and clinical presentation. Presumably, both the disruption of synaptic connections associated with senile plaque formation and the death of tangle-bearing neurons contribute to the syndrome of dementia. There is also the possibility that

neurotoxic fragments of APP can cause neuronal death independently of plaque and tangle formation.

The neurotrophic/neurotoxic properties of β/A4 (or other APP fragments) may account for the secondary neuritic changes that take place around the developing senile plaque. At least some of the PHF-containing dystrophic neurites in the plaque periphery are likely to be connected to tangle-bearing neuronal perikarya, probably via neuropil threads although this has not been examined in detail.

As noted above, neurofibrillary tangles are thought to contain an abnormally phosphorylated fragment of tau protein. It is not yet clear whether abnormal phosphorylation precedes or follows tau deposition, but a likely sequence of events is that exposure to abnormal fragments of APP, possibly β/A4 itself, leads to hyperphosphorylation of tau, which then polymerizes to form stable PHF. While this process may be reversible in its early phases, it becomes irreversible as other modifications of PHF (such as ubiquitination) occur.

One possible route towards abnormal phosphorylation is chronic disturbance of Ca^{2+} homeostasis mediated by the neurotoxic properties of β/A4. The precise molecular details explaining the connection between β/A4 amyloid deposition and neurofibrillary tangle formation is one important focus for further research.

Selectivity of cell death

The above sequence of events provides a basic outline of the processes that lead to plaque and tangle formation and to cell death in AD. However, it is difficult to understand why the distribution of the pathology is not generalized but seems to be centred on the hippocampal/amygdala complex early in the disease, and then seems to spread out from there along neuronal pathways[22].

Too little is known about the distribution, biochemistry and functions of APP or neuronal networks to formulate an hypothesis about the underlying basis for this selective vulnerability. It may be that the disease process starts in the hippocampus because this is an area in which synaptic remodelling (and APP turnover?) occurs to the greatest extent. Alternatively, Yankner *et al.*[47] have recently suggested that selective vulnerability in AD can be explained by a neurotoxic interaction between β/A4 and nerve growth factor. They have stated that β/A4 can induce the production of NGF receptors in certain rat hippocampal neurons, which are then selectively vulnerable to the neurotoxic effects of β/A4.

Whatever the explanation for primary vulnerability, two factors may be important in the subsequent spread of the pathology: electrical signalling along neuronal pathways, or the transfer of pathogenic molecules between neurons. In this respect, the fact that APP is transported along axons may be relevant[16].

A detailed molecular understanding of the pathological cascade outlined above will permit the design of novel drugs to intervene at various points in this process. The recent finding that diffuse plaque formation can be induced in mice by overexpression of APP in transgenic animals[50] may provide one suitable model for testing some of these drugs.

Acknowledgements

We thank Carvell Williams for helpful comments on the manuscript.

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The seminal role of beta-amyloid in the pathogenesis of Alzheimer disease.

Joachim CL, Selkoe DJ.

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New molecular information about Alzheimer disease (AD) is appearing at an unprecedented rate. Much interest centers on the beta A4 amyloid protein, which is progressively deposited in senile plaques and blood vessels in AD brain tissue. The discovery that some kindreds with familial AD have a mutation in the gene coding for the beta A4 amyloid precursor protein (APP) suggests that this mutation alone may be sufficient to cause the full spectrum of clinical and pathological changes that characterize AD. Although APP point mutations may turn out to be relatively rare causes of AD, the idea that accelerated beta A4 deposition is an early and critical event in many patients continues to gain support from studies in humans, animals, and cultured cells. Identification of the biochemical steps leading to production of the beta A4 peptide from APP is now a critical issue. Recent reports indicate that normal lysosomal processing pathways can produce carboxyl-terminal fragments of APP that contain the entire beta A4 sequence, and are therefore potentially amyloidogenic. The mechanisms by which such intermediate forms are further processed and released, resulting in extracellular beta A4 deposits in plaques and vessels, are yet to be determined. It is likely that full elucidation of the beta A4-producing pathways will ultimately yield new therapeutic approaches to this complex and tragic disorder.

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Deposits of amyloid beta protein in the central nervous system o transgenic mice.

Wirak DO, Bayney R, Ramabhadran TV, Fracasso RP, Hart JT, Hauer PE, Hsiau P, Pekar SK, Scangos GA, Trapp BD, et al.

Molecular Therapeutics Inc., Miles Research Center, West Haven, CT 06516

Alzheimer's disease is characterized by widespread deposition of amyloid in the central nervous system. The 4-kilodalton amyloid beta protein is derived from a larger amyloid precursor protein and forms amyloid deposits in the brain by an unknown pathological mechanism. Except for aged nonhuman primates, there is no animal model for Alzheimer's disease. Transgenic mice expressing amyloid beta protein in the brain could provide such a model. To investigate this possibility, the 4-kilodalton human amyloid beta protein was expressed under the control of the promoter of the human amyloid precursor protein in two lines of transgenic mice. Amyloid beta protein accumulated in the dendrites of some but not all hippocampal neurons in 1-year-old transgen mice. Aggregates of the amyloid beta protein formed amyloid-like fibrils tha are similar in appearance to those in the brains of patients with Alzheimer's disease.

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